PLANT PHYSIOLOGY

EDITORS

CHARLES A. SHULL
WALTER F. LOEHWING
BERNARD S. MEYER — †CHARLES B. LIPMAN
GEORGE W. SCARTH — MAX A. MCCALL

D. R. HOAGLAND
EZRA J. KRAUS — EDWIN C. MILLER

VOLUME 19

1944

THE SCIENCE PRESS PRINTING CO.,
LANCASTER, PENNSYLVANIA

TABLE OF CONTENTS

NUMBER 1. JANUARY

The exudation of glutamine from lawn grass ... LAWRENCE C. CURTIS 1– 5
Effect of intermittent irradiation on photoperiodic responses.
R. B. WITHROW and ALICE P. WITHROW 6– 18
Electrodialysis of seeds ... JOHN D. NELLY 19– 32
Effects of oxygen tension on certain physiological responses of rice, barley, and tomato ... J. VLAMIS and A. R. DAVIS 33– 51
Effects of iron on chlorophyllous pigments, ascorbic acid, acidity and carbohydrates of *Ananas comosus* (L.) Merr. supplied with nitrate or ammonium salts.
C. P. SIDERIS and H. Y. YOUNG 52– 75
Rapid analytical methods for some of the more common inorganic constituents of plant tissues ... R. C. LINDNER 76– 89
Evaluation of foliage injury and water loss in connection with use of wax and oil emulsions ... C. L. COMAR and C. G. BARR 90–104
Effect of boron, copper, manganese, and zinc on the enzyme activity of tomato and alfalfa plants grown in the greenhouse.
L. F. BAILEY and J. S. MCHARGUE 105–116
Relation of light to growth of plants.
H. F. THUT and W. E. LOOMIS 117–130
Fruit growth and food transport in cucurbits.
A. S. CRAFTS and O. A. LORENZ 131–138
Relation of weather conditions to onion blast ... LINUS H. JONES 139–147
Semi-micro estimation of reducing sugars ... H. WEINMANN 148–156
Use of root pressures in determining injury to roots by cultivation.
O. A. LEONARD 157–163
Buffer values of the leaves of some plants.
GEORGE E. F. BREWER and EDITH GODAR 164–169
Glass potometers for studies of absorption and exudation by excised roots ... H. F. ROSENE 170–172
Notes ... 173–178

NUMBER 2. APRIL

Apparent photosynthesis in some conifers during winter.
R. O. FREELAND 179–185
Hair sprout of potatoes.
R. B. HARVEY, A. REICHENBERG, BERNICE LEHNER, and P. C. HAMM 186–197
Relation between electrical and curvature responses in the Avena coleoptile to mechanical stimuli ... A. R. SCHRANK 198–211

Science Direct

Some chemical reactions of sulphur dioxide after absorption by alfalfa and sugar beets.
MOYER D. THOMAS, RUSSEL H. HENDRICKS, and GEO. R. HILL 212–226
A study of the sulphur metabolism of wheat, barley and corn using radioactive sulphur.
MOYER D. THOMAS, RUSSEL H. HENDRICKS, LOREN C. BRYNER, and GEO. R. HILL 227–244
Radioautographs showing the distribution of sulphur* in wheat.
BERTRAND F. HARRISON, MOYER D. THOMAS, and GEO. R. HILL 245–257
Ionic absorption by tomato plants as correlated with variations in the composition of the nutrient medium.
KENNETH C. BEESON, C. B. LYON, and MELLE WARD BARRENTINE 258–277
Absorption of carbon dioxide by maize.
J. VERDUIN and W. E. LOOMIS 278–293
Growth responses of hemp to differential soil and air temperatures.
CLARENCE H. NELSON 294–309
Effect of moisture and of treatments with acid and alkali on the rate of formation of free fatty acids in stored cottonseed.
M. L. KARON and A. M. ALTSCHUL 310–325
Composition of fruits and phloem exudate of cucurbits.
A. S. CRAFTS and O. A. LORENZ 326–337
Osmotic quantities in growing cotton bolls.
THOMAS KERR and DONALD B. ANDERSON 338–349
Relation between light intensity and rate of photosynthesis of loblolly pine and certain hardwoods.
PAUL J. KRAMER and JOHN P. DECKER 350–358
The cultivation of Chlorella sp. ..... MILDRED E. MANUEL 359–369
Apparent equilibrium between photosynthesis and respiration in an unrenewed atmosphere.
MOYER D. THOMAS, RUSSEL H. HENDRICKS, and GEO. R. HILL 370–376
Chemical composition of roots and tops of dioecious Lychnis in vegetative and flowering phases of growth.
J. FISHER STANFIELD 377–383
The Hottentot fig as a possible commercial source of tannin.
THOMAS YORT HUM and ROBERTSON PRATT 384–386
Notes 387–390

NUMBER 3. JULY

SIR JOHN RUSSELL ..... FIRMAN E. BEAR 391–393
Concurrency of carbohydrate formation and carbon dioxide absorption during photosynthesis in sunflower leaves.
J. H. C. SMITH 394–403
Some effects of boron supply on the chemical composition of tomato leaflets ..... R. Q. PARKS, C. B. LYON, and S. L. HOOD 404–419

Nutrient interrelations in lime-induced chlorosis.
R. C. LINDNER and C. P. HARLEY 420–439
Mycorrhizal symbiosis in Aplectrum, Corallorhiza and Pinus.
D. T. MACDOUGAL and J. DUFRENOY 440–465
Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots ..... FRANK G. VIETS, JR. 466–480
The growth and carbohydrate responses of *Agropyron smithii* and *Bouteloua gracilis* to changes in nitrogen supply.
H. M. BENEDICT and G. B. BROWN 481–494
Influence of phosphorus supply and the form of available nitrogen on the absorption and the distribution of phosphorus by the tomato plant ..... W. S. BREON, W. S. GILLAM, and D. J. TENDAM 495–506
Sugar movement to roots, mineral uptake, and the growth cycle of the cotton plant ..... FRANK M. EATON and HOWARD E. JOHAM 507–518
Relation of the production of an active emanation to respiration in the avocado fruit ..... HARLAN K. PRATT and J. B. BIALE 519–528
Physical characters of the skin in relation to apple fruit transpiration ..... S. A. PIENIAZEK 529–536
Light and the elongation of the mesocotyl in corn ..... LEWIS H. FLINT 537–543
Cryoscopy of small amounts of expressed tissue sap ..... H. B. CURRIER 544–550
Is natural gas injurious to flowering plants? ..... FELIX G. GUSTAFSON 551–558
Isolation in unialgal culture of lichen gonidia by a simple plasmolysis technique ..... E. R. BOGUSCH 559–561
Use of monochloracetic acid to include ammonia in the "soluble nitrogen" tissue test ..... E. M. EMMERT 562–563
Notes 564–567

NUMBER 4. OCTOBER

Light and pigment development in the Kidney bean.
ROBERT A. WHITMORE 569–578
The growth of *Chlorella pyrenoidosa* under various culture conditions ..... JACK MYERS 579–589
Studies on mineral deficiency in pine ..... CLINTON H. HOBBS 590–602
Sugars in apple and cherry blossoms.
R. B. DUSTMAN and V. B. FISH 603–614
The carotene and ascorbic acid concentration of vegetable varieties.
LEONARD P. PEPKOWITZ, RUSSELL E. LARSON, JOSEPHINE GARDNER, and GERALDINE OWENS 615–626
Spectroscopic stability of chlorophylls *a* and *b* and certain analytical considerations.
F. P. ZSCHEILE, C. L. COMAR, and D. G. HARRIS 627–637
Effect of daylength on growth and reproduction of the cucumber.
L. L. DANIELSON 638–648
Influence of phosphorus supply and the form of available nitrogen on the nitrogen metabolism of the tomato plant.
W. S. BREON and W. S. GILLAM 649–659

The effect of disc thickness on the respiration and the various nitrogen fractions of cut discs of radish roots immersed in water and sugar solutions ..... HUSEIN SAÏD and E. D. H. EL SHISHINY 660–670
Effect of nutrient ratio and concentration on growth and composition of tomato plants and on the occurrence of blossom-end rot of the fruit ..... S. M. RALEIGH and J. A. CHUCKA 671–678
Effect of temperature on photosynthesis and respiration in red and loblolly pines ..... JOHN P. DECKER 679–688
Chlorophyll and carotene content of eighteen tobacco varieties. R. B. GRIFFITH, W. D. VALLEAU, and R. N. JEFFREY 689–693
Changes in chemical composition of tung kernels during germination ..... FREDERICK A. JOHNSTON and HAROLD M. SELL 694–698
A method for measuring root respiration. DAVID G. WHITE and N. F. CHILDERS 699–703
A simple apparatus for sand culture work ..... M. E. PADDICK 704–705
Effect of heavy applications of gypsum on plant growth. W. E. LOOMIS 706–708
Notes 709–716
General index to volume 19 717–732

ERRATA

Page 12 fourth line from bottom, for "270 minute" read 270-minute.
Page 152, delete % in column of ratios, table III.
Page 562, under procedure, line 5, for "$NaClO_4$" read $NaClO_2$.

Vol. 19 No. 1

PLANT PHYSIOLOGY

JANUARY, 1944

THE EXUDATION OF GLUTAMINE FROM LAWN GRASS

LAWRENCE C. CURTIS

(WITH TWO FIGURES)

In 1934 GREENHILL and CHIBNALL (**3**) reported the exudation of glutamine from perennial rye grass when treated with nutrient solutions high in ammonia. This is the first recorded observation of glutamine being exuded from a plant.

FIG. 1. Lawn grass, showing exuded salt, composed largely of glutamine. This salt was confined to the area fertilized, and the paths of the fertilizer spreader are clearly seen.

In April, 1942, a similar phenomenon occurred on a lawn in Hamden, Connecticut. On April 11 an undetermined amount of a 10–5–5 fertilizer, high in ammonium sulphate and potassium chloride was applied to a poor uncut area of a lawn and immediately irrigated with a hose. The weather conditions at the time were cloudless, the nights were cool and no rain fell.

In addition to a heavy dew every night, the grass guttated profusely, and this water remained on the plants well into the middle of the morning. On the day following the fertilizer application, the color of the fertilized area took on a whitish cast which became increasingly more conspicuous for three days. At that time detailed observation showed that a small white deposit was attached to the tip and side of almost every grass blade. The owner of the lawn, thinking that the fertilizer that he had applied had been harmful, asked the Experiment Station for an explanation of this unusual phenomenon.

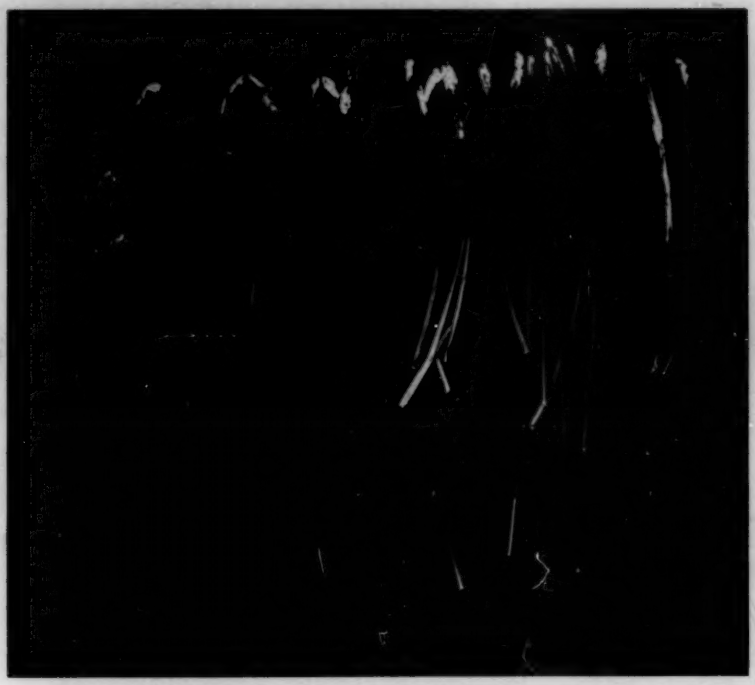


FIG. 2. Individual blades of grass, sorrel, and *Achillea millefolium* all showing exuded salt deposits.

The lawn was indeed an unusual sight when the writer saw it on April 15. Figure 1 clearly shows that the white area corresponded to that part of the lawn where the fertilizer had been applied. The path of the fertilizer spreader is clearly seen. Figure 2 shows individual grass blades, together with specimens of two other species of plants, *Achillea millefolium*, and sorrel, *Rumex acetosella*, bearing the deposits. Two other plant species not included in the picture but which also bore deposits at the leaf margins were clover, *Trifolium repens*, and hawk weed, *Hieracium pratense*.

Identification of material

Samples of the grass with the adhering deposit were brought to the laboratory for identification. Doctors H. B. VICKERY and G. H. PUCHER

of the Biochemical Laboratory of this Station were able, by chemical means, to identify the greater part of the white deposit as glutamine. In a solution prepared by rinsing the sample of cut grass blades with warm water, the equivalent of 6.7 grams of glutamine per kilo of fresh material were found, while the washed clippings contained the equivalent of 5.1 grams per kilo, most of which was located within the blades.

Attempts to reproduce the phenomenon of glutamine exudation have failed. Only traces of glutamine were found in guttation water collected[1] from grass plots which had been treated with a similar fertilizer in an attempt to duplicate the observation. Apparently the delicate balance between the combination of atmospheric conditions, the soil solution as influenced by a suitable nitrogenous fertilizer, and the condition of the plants favorable for the evaporation of the guttation drops are not commonly encountered.

TABLE I

THE FOLLOWING MATERIALS WERE FOUND WHEN THE DEPOSITS FROM 10 GRASS BLADES WERE DISSOLVED IN 5 CC. OF DISTILLED WATER

COMPOUND	AMOUNT P.P.M.
Nitrate nitrogen	Trace
Nitrite nitrogen	Trace
Ammonia nitrogen	5
Phosphorus	4
Potassium	40
Calcium	Trace
Mg	4
Al	0.3
Mn	Trace
Sulphate sulphur	Trace
Chloride	40
pH	4.30

Discussion

Plant physiologists have long recognized the natural process by which water is excreted by the plant—a process called guttation. WILSON (**5, 6**) studied guttation water and listed some of the compounds which he found dissolved in it. He stated his conviction that these compounds came from within the plant by means of a normal physiological process.

The present observation of glutamine formation in plants and its exudation in the guttation water confirms that of GREENHILL and CHIBNALL (**3**), and indicates that this phenomenon is perhaps much more common than has been generally supposed.

It is interesting to note that the plants absorbed large quantities of ammonia, as is evidenced by the amounts of glutamine produced (VICKERY *et al.*, **4**), and such large quantities of potassium and chlorides as are shown in table I. The fact that such small amounts of calcium and other elements

[1] It was found possible to collect large samples of guttation water from grass blades by "mopping" the area with cheese cloth and wringing the cloth into a vessel.

were found in the dried exudate also indicates that these ions must have been present in low concentration in the soil solution.

It is significant that materials absorbed or elaborated by the plant are at times eliminated from the plant in the guttation process. This suggests that perhaps one function of guttation is to eliminate materials that may be temporarily in excess of the requirements of the plant. This does not imply that these materials are responsible for guttation although they may contribute to the process.

The general belief among botanists is that plants do not need an excretory system, comparable to that of animals, to rid themselves of unwanted or unnecessary products of respiration or of synthetic processes (elaboration), or indeed of an excess of substances absorbed from the soil solution by the roots.

The writer contends that most plants apparently do need such a system, and that water absorbed by the roots is the most important material that the plant needs to excrete. There is no delicate balance between the immediate water requirements of the aerial portion of the plant and the amounts absorbed. On bright sunny days the leaves of a plant may wilt because transpiration exceeds absorption and at night the same leaf may lose water in liquid form because the supply exceeds the requirement. The function of guttation seems to be to regulate the turgor pressure within the plant. Any materials present in the guttation water, whether necessary or unnecessary or even toxic to the plant, would be eliminated only because they were present in the vascular system at the particular time that guttation takes place. GREENHILL and CHIBNALL have expressed the belief that possibly the elaborated glutamine might even exercise some selective action on the permeability of the cells and thereby diffuse to the vascular system and eventually to the outside of the leaf. If this assumption be true, then perhaps toxic materials produced by bacteria and fungi in an infected plant might also find their way to the outside of a plant in the same manner.

The fact that chemical compounds reach the outside of the leaf and remain there for varying periods of time is of some importance to those who work with living plants. CURTIS (**1, 2**) has shown that exuded materials may injure the plant leaf directly, as these deposits did these grass blades, and that they may react or combine with spray and dust materials when applied as insecticides or fungicides to increase or decrease their effectiveness or to increase their injury to the host plant.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. CURTIS, L. C. Deleterious effects of guttated fluid on foliage. Amer. Jour. Bot. **30**: 778–781. 1943.
2. ————————. The influence of guttated fluid on pesticides. Phytopath. (In press.)

3. GREENHILL, A. W., and CHIBNALL, A. C. The exudation of glutamine from perennial rye-grass. Biochem. Jour. **28**: 1422–1427. 1934.
4. VICKERY, H. B., PUCHER, G. W., and CLARK, H. E. Glutamine metabolism of the beet. Plant Physiol. **11**: 413–420. 1936.
5. WILSON, J. K. The nature and reaction of water from hydathodes. Cornell Univ. Agr. Exp. Sta. Memoir 65. 1923.
6. ————————. When dew is not dew. The Cornell Rural School Leaflet **20**: 19–36. 1926.

EFFECT OF INTERMITTENT IRRADIATION ON PHOTOPERIODIC RESPONSES[1]

R. B. WITHROW AND ALICE P. WITHROW

(WITH FOUR FIGURES)

Introduction

Intermittent irradiation often has been used as a technique for evaluating the nature of the non-photochemical reactions associated with and limiting the rate of photochemical reactions. Several investigators (**1, 4, 5, 6**) have used intermittently applied radiant energy in studying photoperiodism, but the irradiances used have been far above the point of saturation of the photochemical reaction and little attempt has been made to control the energy relationships per cycle. These high irradiances and unbalanced energies have made it difficult to interpret the results in terms of the interrelationships between the photochemical and the non-photochemical reactions.

This report deals with several series of experiments which were started in the winter of 1938 with the view of determining, by the use of intermittent irradiation, the general interrelationships between the photochemical and non-photochemical reactions associated with photoperiodism. In order to observe quantitatively the influence of the length of the dark period in relation to the effectiveness of the radiant energy in promoting photoperiodic reactions, two conditions were maintained. First, the total energy applied in comparable series was of constant value; and secondly, the energy for the most effective treatment of the series was below the region where photochemical saturation begins. These conditions were used, except in the preliminary experiments where an endeavor was being made to find where saturation of the photochemical reaction begins as measured by phasic responses. Saturation of the photochemical reaction responsible for long photoperiod responses may be said to have occurred at that irradiance beyond which further increases fail to cause comparable increases in photoperiodic effect, *i.e.* vegetativeness in short-day plants or flowering in long-day plants. Below the region of saturation, the photoperiodic effect is approximately proportional to the irradiance (**7**). To date the only measure that we have of the photochemical reaction is the phasic response of the apical meristem and, to some extent, height responses. This is an indirect and unsatisfactory method, but will have to serve until more is known of the photochemical reaction.

General procedure

In all of these experiments, the treatments involved a 24-hour cycle consisting of nine hours of solar irradiation supplemented with 15 hours of incandescent lamp irradiation, applied continuously and intermittently, with varying lengths of dark period. A control short-day treatment was included for each series, with nine hours of solar irradiation and a 15-hour dark period.

[1] Journal Paper no. 98 of the Purdue University Agricultural Experiment Station.

The plants were grown in a greenhouse in a subirrigated bed of gravel and were supplied at regular intervals with a double concentration of the Purdue F-modified nutrient solution (**8**), with the pH kept between 5.5 and 6.5. The supply of nutrients and water was a constant factor, regardless of rate of growth or irradiation treatment.

The solar irradiation period was limited to nine hours by pulling a heavy rubberized curtain, black on the inside and aluminum coated on the outside, over the plants and radiation equipment at 4:30 P.M. and removing it at 7:30 A.M. The bed was divided into sections, 40 by 54 inches, for the individual treatments. The sections were isolated from one another by opaque black rubberized cloth on rollers. These were mounted across the bed and were rolled and unrolled at the time the large curtain was pulled. Each section was irradiated by four tungsten-filament, internal-reflector lamps placed four feet above the gravel surface. The irradiance over each section at 10 cm. above the gravel surface varied plus or minus 5 to 7 per cent. from the values given in the tables.

The lowest irradiances were determined with a Macbeth illuminometer and the other values with both a Macbeth illuminometer and a Weston illuminometer. Both instruments were calibrated against a National Bureau of Standards standard lamp. The lamps used were of such wattage that the irradiance was from ten to twenty per cent. higher than the indicated value, except where noted. The irradiance then was reduced to the correct value by spotting the bulbs with a small brush dipped in a black water-paint, which was made up by adding distilled water to a colloidal graphite paste (Aquadag). It was possible readily to remove or add the paint as necessary to make the proper adjustment in irradiance. The length of the irradiation cycles was controlled by synchronous motor-driven time switches.

In those experiments involving a constant irradiance for all of the intermittent treatments, the same wattage lamp was used for both the intermittent and continuous treatments, even though the irradiance of the intermittent treatment was 20 times that of the continuous when a five per cent. cycle was used. The radiant energy in the continuous treatments was reduced to the correct values by enclosing the lamps in metal shields. In the bottom of each shield was a small opening which was covered with two layers of glass, ground on both surfaces. Thus the color temperature was the same in all treatments and consequently the spectral energy distribution was the same. It was not practical to use the same wattage lamps for all treatments in those experiments in which the duration of the irradiation period for each cycle was held constant, while the irradiance was varied. In such experiments there was a small but probably insignificant variation in spectral energy distribution.

Preliminary experiments

Three preliminary experiments were conducted to determine approximately the irradiances at which saturation of the photochemical reaction be-

gins in various test plants, and its general relation to the length of the dark period.

In the first preliminary experiment, Nobel spinach, China aster (variety Heart of France), and Mukden soybean were subjected to intermittent irradiation on a 30-minute cycle, with the radiant energy applied for five per cent. of the cycle or 1.5 minutes per 30 minutes at 2, 20, and 200 footcandles (fc), resulting in 90, 900, and 9000 footcandle minutes (fcm) per 15-hour period respectively. These were compared with continuous irradiation of 1 and 10 fc, having relative energy values of 900 and 9000 fcm for the 15-hour period of curtaining.

TABLE I

RELATIVE EFFECT OF CONTINUOUS RADIANT ENERGY AND OF INTERMITTENT RADIANT ENERGY APPLIED ON 30-MINUTE CYCLES

IRRADIATION TREATMENTS*	TOTAL SUPPLEMENTAL RADIANT ENERGY	DAYS TO FLOWER		
		SPINACH, NOBEL	CHINA ASTER, HEART OF FRANCE	SOYBEAN, MUKDEN
	fcm†	*days*	*days*	*days*
15-hour dark period	0	.....	.....	36
2 fc 1.5 min./30 min.	90	.....	69	36
20 fc 1.5 min./30 min.	900	24	54	36
200 fc 1.5 min./30 min.	9000	20	54	.....
1 fc Continuous	900	20	54	.....
10 fc Continuous	9000	20	54	.....

* 20 plants per treatment.
† Footcandle minutes.

The night temperature was about 55° F. for the first and second preliminary experiments. China aster is temperature-sensitive in its flowering response, but it is a long-day plant at the temperatures used (**2, 3**).

Observations were made at intervals of from one to four days for macroscopic flower buds in all of these studies. The time given in the tables for flowering is, in most cases, that time at which over 90 per cent. of all the plants in the treatment had shown macroscopic flower buds. In cases where macroscopic buds failed to develop, micro-dissection methods were employed.

The results given in table I indicate that 9000 fcm, applied as either continuous or intermittent radiant energy on a 30-minute cycle, was well in the region of saturation for the plants used. Nine hundred fcm produced saturation for continuous irradiation, but the intermittent application of the same total energy indicated that the efficiency was less and that this value of energy was approaching saturation for spinach and Mukden soy-

bean. Ninety fcm intermittent radiant energy was below saturation for spinach and soybean, although aster formed flower buds.

On the basis of these results, a series of irradiances of intermittent radiant energy was used, including 2, 4, 8, 16, 32, and 64 fc on a 30-minute cycle, in which the irradiation period was 10 per cent. of the cycle or 3 minutes. These were compared with a 0.4 fc continuous treatment which had the same fcm value as the 4 fc intermittent treatment. The plant material and number of plants per treatment were Nobel spinach (22 plants), dill (25 plants), China aster (variety Heart of France) (10 plants), Xanthium (20 plants), and Biloxi soybean (20 plants). The results for flowering time are plotted in figure 1. Using the sequence of flower bud formation as a

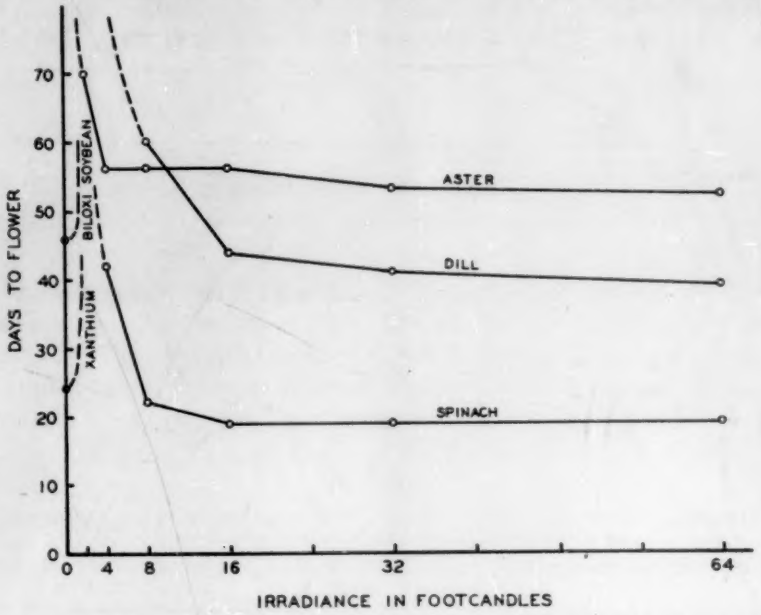


FIG. 1. Flowering time under the various irradiances of intermittently applied radiant energy. Broken lines indicate probable shapes of curves at low irradiances.

measure of the photochemical reaction, it appeared that saturation had been reached for all of the long-day plants at 16 fc intermittent irradiation, which corresponds to 1.6 fc continuous or 1440 fcm. The reaction for aster began to saturate at about 4 fc intermittent, and for spinach at 8 fc. All of the values of irradiance used were sufficiently high to completely inhibit flowering of both Xanthium and soybean, the two short-day plants. Similar saturation curves were obtained when the height data were plotted as is shown in figure 2. With the exception of dill, saturation in height had occurred for the plants at 16 fc of intermittent irradiation. Dill required about 32 fc for saturation.

In the third preliminary experiment, Nobel spinach and Biloxi soybean were used. Radiant energy was applied so as to produce two relative energy

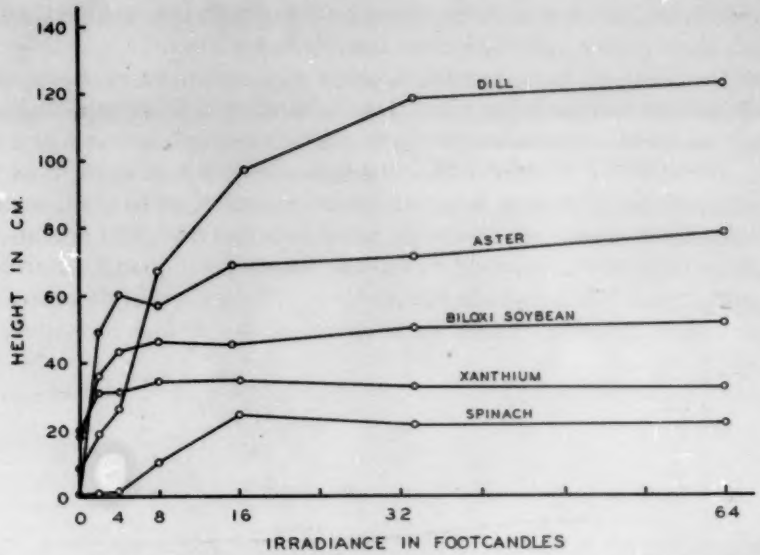


FIG. 2. Height of the plants under the various irradiances of intermittently applied radiant energy.

levels of 90 and 900 fcm. Each of these energy levels was applied as follows: (1) for six minutes at the end of 7½ hours so that one irradiation period occurred in the middle of the 15-hour curtaining period; (2) for six minutes per hour so that 15 irradiation periods occurred per 15 hours; and (3) as continuous irradiation. The irradiances were adjusted as indicated in table II.

TABLE II

EFFECT OF VARYING IRRADIANCE AND LENGTH OF DARK PERIOD ON FLOWERING OF NOBEL SPINACH AND BILOXI SOYBEAN

IRRADIATION TREATMENTS*	TOTAL SUPPLEMENTAL RADIANT ENERGY	DAYS TO FLOWER	
		SPINACH, NOBEL	SOYBEAN, BILOXI
	fcm	*days*	*days*
15-hour dark period	0	.....	25†
15 fc 6 min./15 hr. at 7½-hr. intervals	90	.....	30†
1 fc 6 min./hr. at hourly intervals	90	15% at 70 days	90% at 70 days
0.1 fc Continuous	90	20% at 70 days	64% at 70 days
150 fc 6 min./15 hr. at 7½-hr. intervals	900	.....	45†
10 fc 6 min./hr. at hourly intervals	900	17†	.....
1 fc Continuous	900	14†	.....

* 40 plants per treatment.
† Macroscopic buds.

In comparison with continuous irradiation, the results at 90 fcm intermittent show that a dark period of nearly one hour results in a slight decrease in the efficiency of the radiant energy in inducing flower bud initiation in spinach and delaying it in Biloxi soybean. The efficiency drops off very markedly with a dark period of 7½ hours. The results at 900 fcm were similar but the differences were not as great, since 900 fcm was approaching saturation. Figure 3 shows spinach taken from the various treatments in this third preliminary experiment. The spinach plants receiving one six-minute period of irradiation per 15 hours were strictly vegetative and similar in form to those given one 15-hour dark period out of each 24-hour photoperiod cycle.

These preliminary experiments indicate that the photoperiodic responses began to saturate at irradiances in the region of 2 fc, or less, for continuous

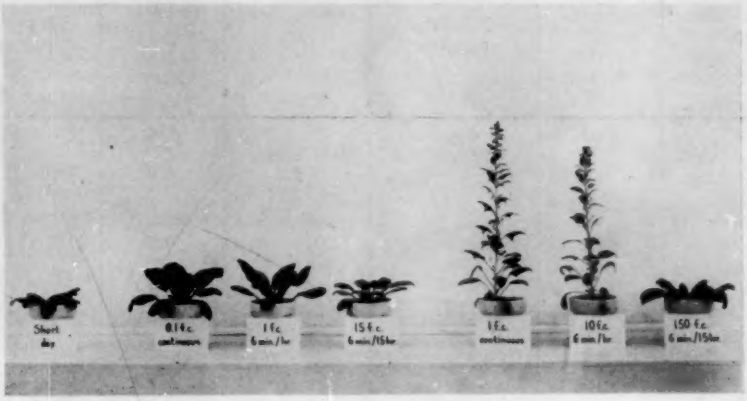


FIG. 3. Effect of varying supplemental irradiance and length of dark period on the growth and flowering of Nobel spinach.

irradiation, that intermittent radiant energy was less efficient than the same total energy applied continuously, and that the efficiency decreased as the length of the dark period was increased.

Final experiments

The final experimentation was confined to Nobel spinach and Biloxi soybean. In each experiment, the intermittent treatments and one continuous irradiation treatment involved approximately the same total relative energy in fcm. One continuous treatment with half this energy was included in each experiment so as to determine approximately that length of dark period at which the efficiency of the radiant energy was 50 per cent. of the efficiency of the continuous radiant energy.

The intermittent treatments involved the application of radiant energy on cycles of 3.3, 10, 30, 90, 180, and 270 minutes. The control treatment consisted of a 15-hour (900-minute) dark period. The intermittent radiant energy was applied in two ways: (1) a constant length of irradiation period

of 10 seconds for each cycle, with the irradiance increasing in direct proportion to the length of the cycle so that the same total relative energy was applied in all treatments; and (2) a constant irradiance for each treatment, with the length of the irradiation period increased in direct proportion to the length of the cycle and amounting to five per cent. of the cycle. These two types of cycle were included in order to make certain that the results secured were due to variations in the length of the intervening dark period and not to the method of application of the radiant energy.

TABLE III

FLOWERING RESPONSE OF NOBEL SPINACH TO INTERMITTENT IRRADIATION ON CONSTANT TIME AND CONSTANT IRRADIANCE CYCLES

LENGTH OF CYCLE, IRRADIANCE, AND LENGTH OF IRRADIATION PERIOD*	TOTAL SUPPLEMENTAL RADIANT ENERGY	DAYS TO FLOWER	LENGTH OF CYCLE, IRRADIANCE, AND LENGTH OF IRRADIATION PERIOD†	TOTAL SUPPLEMENTAL RADIANT ENERGY	DAYS TO FLOWER
	fcm	*days*		*fcm*	*days*
900 min. 0 fc	0	.....	900 min. 0 fc	0	.....
180 min. 540 fc 10 sec.	450	50% at 50 days	270 min. 10 fc 810 sec.	405	41% at 33 days
90 min. 270 fc 10 sec.	450	40	90 min. 10 fc 270 sec.	450	33
30 min. 90 fc 10 sec.	450	35	30 min. 10 fc 90 sec.	450	29
10 min. 30 fc 10 sec.	450	35	10 min. 10 fc 30 sec.	450	29
3.3 min. 10 fc 10 sec.	450	32	3.3 min. 10 fc 10 sec.	450	26
Continuous 0.5 fc	450	31	Continuous 0.5 fc	450	25
Continuous 0.25 fc	225	58% at 50 days	Continuous 0.25 fc	225	30

* Irradiation period of each cycle was held constant at 10 sec. with exception of the control and continuous treatments. Irradiance was varied to give equal total energies per 15-hour period of curtaining; 80 plants per treatment.

† Irradiance was held at 10 fc and irradiation period was 5 per cent. of the cycle, with exception of control and continuous treatments; 65 plants per treatment.

In table III are given the results of two comparable experiments with spinach, with the total relative energy of 450 fcm applied on constant time and on constant irradiance cycles. In the constant irradiance series, the total energy for the 270 minute (4.5-hour) cycle was 405 fcm since it was possible to have only three irradiation periods during the period of curtaining. The results indicate that essentially the same relationships are obtained with both methods of application of radiant energy, even though the actual

irradiance on the constant time cycle varied as much as 54 times that of the constant irradiance cycles. As the length of the dark period was increased, the time required for flower bud appearance was increased for spinach. The increase was only slight as the dark period was increased up to 90 minutes, but it was very marked beyond 90 minutes. The efficiency in the utilization of the radiant energy falls off very markedly after 90 minutes. It drops to 50 per cent. at a length of dark period between 90 and 180 minutes on the constant time cycle. The length of dark period for 50 per cent. efficiency is between 30 and 90 minutes for the constant irradiance cycle. At periods

TABLE IV

FLOWERING RESPONSE OF NOBEL SPINACH AND BILOXI SOYBEAN TO INTERMITTENT IRRADIATION ON CONSTANT IRRADIANCE CYCLES

LENGTH OF CYCLE, IRRADIANCE, AND LENGTH OF IRRADIATION PERIOD*	TOTAL SUPPLEMENTAL RADIANT ENERGY	DAYS TO FLOWER		
		SPINACH	SOYBEAN	
			I	II
	fcm	*days*	*days*	*days*
900 min. 0 fc	0	.....	17	17
270 min. 4 fc 810 sec.	162	68% at 62 days	43	30
90 min. 4 fc 270 sec.	180	39	53% at 50 days	.....
30 min. 4 fc 90 sec.	180	31	.....	.....
10 min. 4 fc 30 sec.	180	29	.....	.....
3.3 min. 4 fc 10 sec.	180	29	.....	.....
Continuous 0.2 fc	180	29	.....	.....
Continuous 0.1 fc	90	93% at 62 days	53% at 50 days	44% at 40 days

* Irradiance was held constant at 4 fc and irradiation period was 5 per cent. of the cycle, with exception of control and continuous treatments; 80 plants per treatment.

longer than 90 minutes, it was necessary to use micro-dissection methods to secure final flowering data as no flower stalks were formed. With these long periods, not only was flowering greatly delayed, but flower bud initiation failed to occur in a large proportion of the plants.

In the second series of experiments, Biloxi soybean as well as spinach was used, and it was necessary to use lower values of total energy since, in Biloxi soybean, the photoperiodic mechanism is saturated at a lower irradiance than in spinach. The radiant energy was applied at a constant irradiance of 4 fc, with the time of application 5 per cent. of the cycle, totalling

180 fcm relative energy except for the 270 minute cycle, which had a total relative energy of 162 fcm. The continuous irradiation was at 0.2 and 0.1 fc. The experiment was run at two different times with soybean, series I during the winter and series II during the spring months.

The results are given in table IV. For spinach, they were similar to those obtained previously with a higher energy level and show that the length of the dark period for 50 per cent. efficiency is between 90 and 270 minutes.

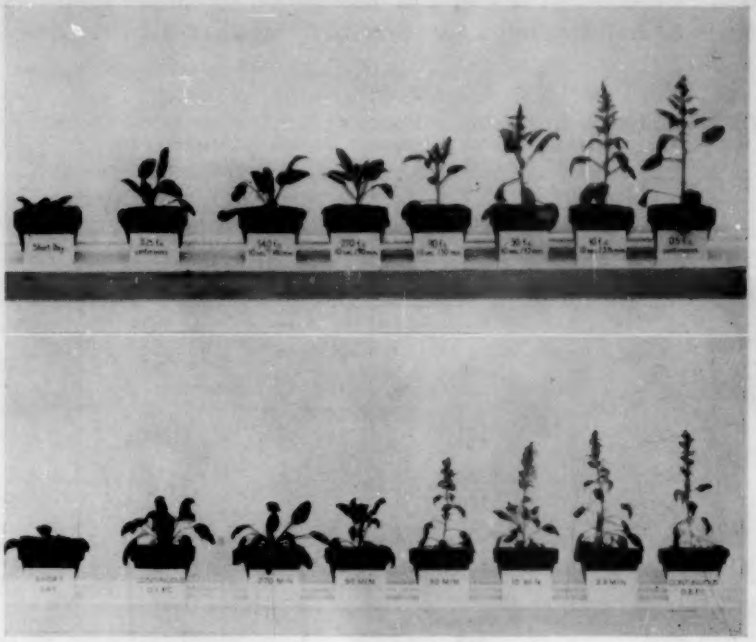


FIG. 4. Flowering response of Nobel spinach to intermittent irradiation on constant time (upper) and constant irradiance (lower) cycles.

Upper row, left to right:

1. Short day.
2. 0.25 fc continuous.
3. 540 fc, 10 sec. per 180 min.
4. 270 fc, 10 sec. per 90 min.
5. 90 fc, 10 sec. per 30 min.
6. 30 fc, 10 sec. per 10 min.
7. 10 fc, 10 sec. per 3⅓ min.
8. 0.5 fc continuous.

Lower row, left to right:

1. Short day.
2. 0.1 fc continuous.
3. 270 min.
4. 90 min.
5. 30 min.
6. 10 min.
7. 3⅓ min.
8. 0.2 fc continuous.

Soybean gave similar results in that the length of the dark period for 50 per cent. efficiency of the radiant energy was at 90 minutes in the first experiment and between 90 and 270 in the second. Micro-dissection methods were used in obtaining final flowering data. In cycles varying from 0 (continuous irradiation) to 30 minutes, all of the soybean plants were strictly vegetative as evidenced by the lack of floral primordia at the end of the experiment.

It should be noted that direct comparisons of data cannot be made between the various series of the preliminary and final experiments since they

were conducted at different seasons of the year and different lots of seed were used during the four-year period of experimentation.

Discussion

The interpretation of these results with intermittently applied radiant energy is especially difficult because no direct method of measuring the rate of the photochemical reaction taking place in the leaves is available. The reaction was measured indirectly, in most cases by observing the responses of the apical meristems in terms of the time required for vegetative meristems to form flower buds. Considering that the leaf is spatially removed from the bud by the petiole and a length of stem, it is obvious that the method of measuring the photochemical reaction is very indirect and the results cannot be interpreted with certainty as due to factors limiting directly the rate of the photochemical reaction. It is quite conceivable that the limiting factors may be concerned with limitations in the rate of transport of the stimulus to the bud, to loss in stimulus as a result of secondary reactions, or to the incapacity of the meristem to respond to stimuli above a very low intensity. For reasons which will appear later, the following discussion is based on the assumption that the limitations are concerned primarily with the photochemical reaction.

The responses secured with intermittent irradiation may be explained on the theory that: (1) the substance which is photoactivated is formed by non-photochemical reactions at a relatively slow rate; and (2) that this substance attains a very low equilibrium concentration in darkness. The assumption that the rate of the non-photochemical reaction is slow is based upon the irradiance data which indicates that saturation of the long-day responses occurs at very low irradiances. For the plants used, the rate of formation of the substance to be photoactivated appears to limit the rate of the photochemical reaction at irradiances much above 1 fc of continuous irradiation, and these low irradiances appear sufficient to photoactivate the substance as fast as it is formed. Increases in irradiance, therefore, do not result in proportionate increases in photoactivation.

Since the efficiency of the radiant energy in producing long-day reactions is decreased as the length of the dark period is increased, it appears that the products of the associated non-photochemical reactions are not cumulative beyond a certain low equilibrium concentration. If during a long dark period the substance could accumulate at an approximately constant rate in the leaf, the same quantity of photochemical product could conceivably be obtained within a few minutes at the beginning of a period of high solar irradiance, regardless of the length of the intervening dark period in the 24-hour cycles used in these experiments. Since this is not so, it would appear that a low equilibrium concentration is reached, beyond which no further increase can take place. The equilibrium concentration probably is nearly reached in a dark period of from 90 to 180 minutes for Nobel spinach and Biloxi soybean.

This conclusion regarding a low equilibrium concentration is further substantiated by the fact that, to our knowledge, no pigment has been extracted from the leaf which has absorption bands which coincide with the spectral characteristics of the response. It has been shown that it is the longer wavelengths of the visible spectrum between 6000 and 7000 A which are primarily effective in causing the long-day response in long- and short-day plants (**7, 9**). This implies that, unless a strong filtering action occurs, the photoactivated substance is a pigment having its strongest absorption bands in the orange-red region of the spectrum, with relatively weak bands elsewhere. If the equilibrium concentration for such a pigment were relatively high, it is unlikely that it would have escaped detection.

It is possible to explain the kinetics of photoperiodism on the basis of these two postulated relationships limiting the photochemical reaction. Under natural conditions, in the temperate zone, plants are exposed to periods of solar irradiation in which the irradiance seldom if ever drops below 100 fc, which is well above the bend of the saturation curve. These periods of irradiation are followed by a relatively long night which may be considered as a dark period. The relative duration of the periods of irradiation and darkness in a 24-hour cycle determine the flowering responses of many plants. If the photochemical reaction were not limited by the slow rate of formation of the substance to be photoactivated, a large photochemical product would be formed during a relatively short period of high irradiation, even if the day were very short. On the other hand, if the equilibrium concentration were high, the non-photochemical product could accumulate to relatively high values during a long dark period and such accumulation would allow a high rate of photochemical reaction during the initial period of irradiation, which would more than compensate for a slow rate of the non-photochemical reaction. It is obvious, then, that a relatively large photochemical product could be formed if either of these limiting factors were not operative. On the basis of this type of reasoning, without either of these two limiting factors, photoperiodism could not exist.

While the authors fully realize that this theoretical discussion is based upon many unverified assumptions, it offers a relatively simple explanation of the kinetics of photoperiodism and may serve as a basis for further experimentation.

Summary

Several series of experiments are described in which intermittent irradiation was used to determine the interrelationships between the photochemical and non-photochemical reactions associated with photoperiodism. The test plants used in the preliminary experiments were Nobel spinach, China aster, dill, Xanthium, Mukden soybean, and Biloxi soybean. In the final experimentation, Nobel spinach and Biloxi soybean were used. The intermittent irradiation was used in all cases to supplement nine hours of solar radiant energy. In all the investigations except the irradiance series, the total supplemental radiant energy employed in any comparable series was of a con-

stant value and, in the final series, the energy employed for the most effective treatment of the series was slightly below the value at which photochemical saturation began. Two types of final experiments are described: (1) those involving constant values of irradiance, the length of time of application varying directly with the length of the intervening dark period; and (2) those involving a constant length of time of supplemental irradiation, with the irradiance varying directly with the length of the intervening dark period.

The results show that: (1) the region of saturation of the photochemical reaction for most of the plants used is in the neighborhood of one footcandle, since further increases in irradiance failed to bring about corresponding increases in the photoperiodic responses; and (2) the greater the length of the dark period between irradiation cycles, the less the efficiency of the radiant energy in promoting the long-photoperiod responses, with the efficiency falling off rapidly when the dark periods were around 90 minutes or longer for spinach and Biloxi soybean. In all cases, continuous irradiation resulted in the highest efficiency.

From these results, a theory has been postulated concerning the kinetics of the photoperiodic reaction based on two relationships which appear to limit the photochemical reaction; namely, (1) the relatively slow rate of the non-photochemical reaction which forms the substance to be photoactivated; and (2) the relatively low equilibrium concentration which this substance attains during long periods of darkness.

PURDUE UNIVERSITY
WEST LAFAYETTE, INDIANA

LITERATURE CITED

1. ALLARD, H. A., and GARNER, W. W. Responses of some plants to equal and unequal ratios of light and darkness in cycles ranging from 1 hour to 72 hours. Jour. Agr. Res. **63**: 305–330. 1941.
2. BIEBEL, J. P. Temperature, photoperiod, flowering and morphology in cosmos and China aster. Proc. Amer. Soc. Hort. Sci. **34**: 635–643. 1937.
3. ————, GREENE, L., and WITHROW, R. B. Flowering response to temperature in China aster. Proc. Amer. Soc. Hort. Sci. **33**: 645–646. 1936.
4. GARNER, W. W., and ALLARD, H. A. Effect of short alternating periods of light and darkness on plant growth. Science n.s. **66**: 40–42. 1927.
5. ————, and ————. Effect of abnormally long and short alternations of light and darkness on growth and development of plants. Jour. Agr. Res. **42**: 629–651. 1931.
6. HAMNER, K. C. Interrelation of light and darkness in photoperiodic induction. Bot. Gaz. **101**: 658–687. 1940.

7. WITHROW, R. B., and BENEDICT, H. M. Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wavelength of artificial light used to lengthen the daylight period. Plant Physiol. **11**: 225–249. 1936.

8. ————, BIEBEL, J. P., and EASTWOOD, T. M. Nutrient solution culture of greenhouse crops. Indiana Agr. Exp. Sta. Cir. **277**. 1943.

9. ————, and WITHROW, ALICE P. The effect of various wavebands of supplementary radiation on the photoperiodic response of certain plants. Plant Physiol. **15**: 609–624. 1940.

ELECTRODIALYSIS OF SEEDS

JOHN D. NELLY

(WITH TWO FIGURES)

Introduction

The study herein reported was undertaken with the purpose of learning something of the effects of electrolysis on a living organism. When electrolysis takes place in living tissues in the presence of membranes the process can more properly be called electrodialysis. The earliest work on the electrodialysis of plant material was that of LISBONNE and VULQUIN (**3**, **4**) in which they reported the inactivation of malt amylase after electrodialysis at 110 volts d.c. under sterile conditions for periods of five to twelve days. Treatment of this inactivated amylase with such salts as NaH_2PO_4, Na_2HPO_4, and KNO_3 partially restored its hydrolyzing power. Before the work of LISBONNE and VULQUIN it was thought that animal and plant amylases were essentially different because animal amylases could be inactivated by simple dialysis while plant amylases did not lose their hydrolytic power when subjected to the same process. The essential difference lay in the fact that plant amylases needed something more powerful than ordinary dialysis to free them of their electrolytes.

The conventional type of electrodialysis cell consists of a cell divided into three compartments by means of two differentially permeable membranes. Electrodes are placed in the end compartments and the suspension to be electrodialyzed is placed in the middle compartment. Electrolytes which can pass through the membrane pass out into the end chambers under the influence of the electrical potential. Ions with positive charges on them go to the cathode chamber while negative ions pass into the anode chamber. A colloidal suspension may be purified in this manner by the process of losing electrolytes to the anode and cathode compartments of the electrodialysis cell while the colloidal material remains in the middle chamber. This method may be used to free gelatin of most of its inorganic matter and to remove adsorbed bases from clay. In the case of living tissues such structures as cell membranes and seed coats determine which electrolytes shall pass through them.

Diastase from pancreatic juice of the cow was electrolyzed by MAIGNON (**6**) in a 16-mm. U-tube at 110 volts d.c. for as long as six days. Prolonged dialysis resulted in a loss of diastatic activity, which MAIGNON attributed to dissociation of organo-mineral groupings in the diastase and not to the formation of such substances as hypochloric or persulphuric acids at the electrodes. MOORE, REEVES, and HIXON (**10**) used electrodialysis in a study of Jonathan spot of apples. They claimed that the dialysate of apple parings with Jonathan spot was higher in basic than acidic constituents, while that from normal apple parings showed equivalent amounts of acid and base. A

study of the effects of electrodialysis has been published by MULLISON (**11**), in which he reported loss of ash constituents from the seeds, decreased germination, and stunted growth of plants from electrodialyzed Alaska pea seeds. Electrodialysis was employed by MAKSIMOW (**7**) on legume seeds, grain seeds, oil seeds, and tobacco. He showed that partial demineralization reduced the germination of legume and oil seeds but not of grain seeds. SEIFRIZ (**13**) has demonstrated the breakdown of plant tissue under the influence of an electric current. He placed the tissue under consideration in a dish partially filled with 0.1 N sodium chloride solution, inserted a platinum electrode into the tissue, and had the other platinum electrode in the surrounding sodium chloride solution. Through this he allowed a 110-volt direct current to pass for 18–24 hours. When the cathode was in the tissue a very marked breakdown of the tissue occurred along with a great increase in pH in the vicinity of the electrode. When the anode was in the tissue, breakdown was slight and the pH was not lowered to any great extent. SEIFRIZ attributed breakdown at the cathode to autolysis favored by reducing conditions. After autolysis oxidation would be favored by the highly alkaline condition of the cathode.

Materials and methods

Electrodialysis was carried out in a Mattson electrodialysis cell, using parchment paper membranes, a copper grid cathode, and a carbon anode. A direct current of 80 to 150 volts was supplied by either a rectifier or a direct current line. The rectifier was equipped with a rheostat for regulating voltage, while the voltage from the direct current line was controlled by means of a resistance coil arranged in a circuit as a potentiometer. Temperature of the cell was controlled by running water through glass coils placed in the end chambers. Although no attempt was made at rigid control of the temperature, this cooling system kept the temperature of the cell near 24° C. Before being placed in the cell the seeds were given a preliminary soaking for either five or six hours in enough distilled water to allow maximum swelling for that length of time. During electrodialysis the seeds were suspended in a cheesecloth bag in the middle chamber of the cell. After the cell was filled with distilled water and the seeds placed in position a steady potential was maintained until the current increased to 450 milliamperes. When the current approached this value, it was turned off and the liquid in the end chambers was replaced with distilled water.

Subsequent to treatment seeds were placed in flannel roll type seed testers and kept at 20° C. for germination. Germination counts were made at the end of either three or five days.

Carbon dioxide production of germinating seeds was determined by absorption in barium hydroxide in Pettenkoffer tubes.

For mineral analyses the seeds were ashed whole in large crucibles in a muffle furnace at temperatures between 500° and 550° C. Seeds which had been electrolyzed for periods of an hour or longer were extremely hard to

ash and had to be treated with nitric and hydrochloric acids to induce ashing. Apparently the ashing process is catalyzed by the presence of some substance or substances lost during the electrodialysis process. After dissolving the ash in hydrochloric acid it was used in the determination of magnesium, calcium, and potassium. Calcium was determined volumetrically by precipitating as the oxalate and titrating with standard potassium permanganate. For magnesium the gravimetric method of weighing magnesium pyrophosphate was employed. The perchloric acid method was used in the determination of potassium (**14**). Before the potassium determinations were made, such interfering substances as aluminum, calcium, phosphates, and sulphates were removed by the method described by SCOTT (**12**).

Ascorbic acid was determined in the germinating seeds by the titration method, using the dye 2,6 dichlorophenolindophenol. Extracts from the seeds were made by triturating them with ignited acid-washed sand in a solution of 3 per cent. trichloracetic and 2 per cent. metaphosphoric acid. This method of determining ascorbic acid is outlined in full by MACLINN and FELLERS (**5**). Standardization of the dye was carried out by the method of MENAKER and GUERRANT (**9**), using sodium thiosulphate.

Results

ELECTRODIALYSIS AND GERMINATION

One of the most striking effects of electrodialysis on seeds is the inhibition of germination. Seeds were soaked in distilled water before being placed in the electrodialysis cell. The Laxton's Progress pea seeds were given a preliminary soaking of five hours, while the Alaska peas and red kidney bean seeds were given a preliminary soaking of six hours. After the period of treatment, both control and test seeds were placed in flannel roll type seed testers soaked in tap water and were kept at 20° C. for germination. The effects of electrodialysis on the germination of pea and red kidney bean seeds are summarized in tables I to IV.

Seeds which failed to germinate, whether electrodialyzed or control seeds, were always infected with bacteria and sometimes with fungi. Con-

TABLE I

EFFECT OF ELECTRODIALYSIS AT 150 VOLTS ON THE GERMINATION OF LAXTON'S PROGRESS PEA SEEDS

PERIOD OF TREATMENT	CONTROL		ELECTRODIALYSIS	
	NUMBER OF SEEDS USED	PERCENTAGE OF GERMINATION	NUMBER OF SEEDS USED	PERCENTAGE OF GERMINATION
min.		%		%
15	80	90	80	59
30	223	81	224	26
60	223	83	224	18
90	203	93	200	7
120	21	86	20	0
180	140	85	140	2

TABLE II

EFFECT OF ELECTRODIALYSIS AT 110 VOLTS ON THE GERMINATION OF ALASKA PEA SEEDS

SAMPLE NUMBER	PERCENTAGE OF GERMINATION					
	1 HOUR		2 HOURS		3 HOURS	
	CONTROL	ELECTRO-DIALYSIS	CONTROL	ELECTRO-DIALYSIS	CONTROL	ELECTRO-DIALYSIS
	%	%	%	%	%	%
1	84	62	83	51	90	27
2	94	66	90	47	92	32
3	80	50	84	16	86	29
Number of seeds used	330	330	330	330	330	330

TABLE III

EFFECT OF ELECTRODIALYSIS AT 80 VOLTS ON THE GERMINATION OF ALASKA PEA SEEDS

SAMPLE NUMBER	PERCENTAGE OF GERMINATION					
	1 HOUR		2 HOURS		3 HOURS	
	CONTROL	ELECTRO-DIALYSIS	CONTROL	ELECTRO-DIALYSIS	CONTROL	ELECTRO-DIALYSIS
	%	%	%	%	%	%
1	91	39	83	33	84	7
2	78	47	86	35	89	12
3	82	51	90	31	81	7
Number of seeds used	330	330	330	330	330	330

TABLE IV

EFFECT OF ELECTRODIALYSIS AT 80 VOLTS ON THE GERMINATION OF RED KIDNEY BEAN SEEDS

SAMPLE NUMBER	PERCENTAGE OF GERMINATION			
	1 HOUR		2 HOURS	
	CONTROL	ELECTRO-DIALYSIS	CONTROL	ELECTRO-DIALYSIS
	%	%	%	%
1	93	26	98	13
2	84	25	98	8
3	95	37	94	4
4	89	20	81	9
5	.....	.....	87	26
6	.....	.....	94	6
Number of seeds used	220	220	330	330

trol lots never had fungi growing on the sprouted seeds. Presence of fungi on sprouted electrodialyzed seeds was somewhat common. Thus it can be seen that some change which takes place during electrodialysis makes the seed a better growth medium for fungi.

Effects of electrodialysis on carbon dioxide production

Because of the fact that electrodialysis had such a marked effect on germination it was decided that a study should be made to learn whether or not this reduced germination was accompanied with a change in respiratory activity. For these determinations weighed samples of about sixty seeds of Laxton's Progress pea were used. Each lot of seeds was soaked for five hours in 100 ml. of distilled water at room temperature before it was placed in the electrodialysis cell. When the sample to be electrodialyzed was placed in the cell the water in which the control sample had been soaking was poured off and fresh water added to the beaker containing the control seeds.

For this part of the work the seeds were electrodialyzed at 150 volts. At the end of the dialysis period the seeds were placed in the respiration apparatus in order to determine their rates of carbon dioxide production. The time during which the seeds were left in the carbon dioxide apparatus was usually about one and one-quarter hours. Production rate of carbon dioxide is expressed in milligrams per hour per gram of seed. The weight basis for this expression is the air dry weight of the seeds at the beginning of the experiment. After the initial carbon dioxide determination the seeds were placed in flannel rolls moistened in tap water and kept at 20° C. The seeds were removed from the rolls long enough each following day to determine their rates of carbon dioxide production.

Seeds which are treated by the electrodialysis process are especially susceptible to attack by microorganisms. It is conceivable that these microorganisms might greatly alter the carbon dioxide production. At the end of 24 hours there was no evidence of infection, but it could be noticed on the second day after electrodialysis. In order to discourage growth of the infecting organisms, both treated and control seeds were soaked 3 minutes in a 0.25 per cent. sodium hypochlorite solution and rinsed in tap water before being placed in the respiration chambers on the second and following days. By this treatment the seeds were kept in a sound condition; otherwise, they would have been disintegrated at the end of a few days.

Rates of carbon dioxide production for the variously treated seeds are indicated in table V. The means of the results presented in table V are plotted in figure 1. A marked difference in carbon dioxide production of electrodialyzed and control seeds is seen. Together with this lesser carbon dioxide production is found a marked decrease in the germinating percentage of the seeds. Germination, however, is not directly proportional to carbon dioxide production of the seeds. For instance, the seeds electrodialyzed 30 minutes have about thirteen times the germination percentage of seeds treated for 180 minutes, while the carbon dioxide production of the

former never becomes much more than twice that of the latter. It seems that seeds lose their ability to germinate but can not be killed because they still retain their respiration in part. Both the 90-minute and the 180-minute control lots of seeds exhibit much higher initial rates of respiration than did the 30-minute and 60-minute controls. This may possibly be explained by the fact that they were in water for longer periods than the other controls

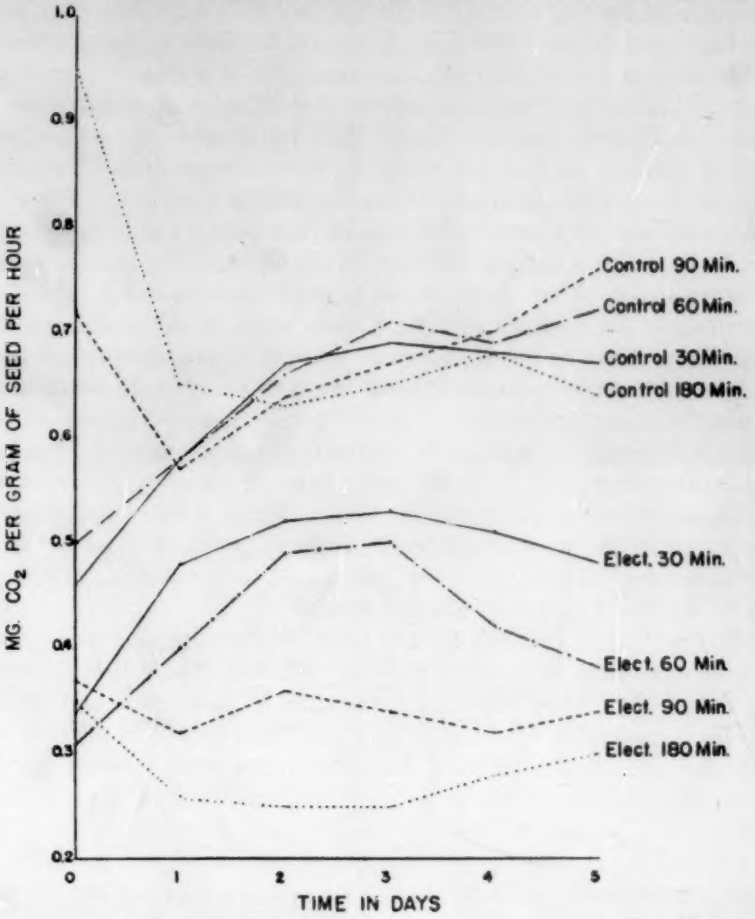


FIG. 1. Graphical representation of effects of electrodialysis on carbon dioxide production of pea seeds. Curves were plotted from means of results presented in table V.

and that increased moisture content may account for the higher respiratory rate. It is known that respiration in seeds increases with an increase in moisture content.

AMOUNTS OF ACID AND ALKALI REMOVED IN ELECTRODIALYSIS

In MATTSON's work (8) with the electrodialysis of soils and in the work of MOORE, REEVES, and HIXON (10) on the electrodialysis of apple parings,

TABLE V

RATES OF CARBON DIOXIDE PRODUCTION IMMEDIATELY AFTER AND AT ONE- TO FIVE-DAY INTERVALS AFTER ELECTRODIALYSIS. SEEDS WERE ELECTRODIALYZED FOR PERIODS OF 30, 60, 90, AND 180 MINUTES. BOTH CONTROL AND ELECTRODIALYZED SAMPLES WERE RUN IN DUPLICATE OR TRIPLICATE. DETERMINATIONS WERE MADE AT 20° C.

TIME PERIODS	MG. CO_2 PER GM. OF SEED PER HOUR							
	30 MINUTES		60 MINUTES		90 MINUTES		180 MINUTES	
	CONTROL	ELECTRODIALYSIS	CONTROL	ELECTRODIALYSIS	CONTROL	ELECTRODIALYSIS	CONTROL	ELECTRODIALYSIS
Beginning	0.47	0.37	0.47	0.33	0.74	0.40	0.96	0.35
	0.47	0.34	0.49	0.28	0.74	0.35	0.93	0.35
	0.44	0.32	0.54	0.33	0.69	0.35	.....	.....
1st day	0.59	0.49	0.55	0.40	0.54	0.33	0.65	0.27
	0.60	0.50	0.56	0.36	0.60	0.32	0.64	0.24
	0.54	0.44	0.64	0.43	0.57	0.32	.....	.....
2nd day	0.71	0.51	0.63	0.47	0.65	0.34	0.64	0.26
	0.65	0.51	0.65	0.48	0.63	0.36	0.62	0.23
	0.66	0.55	0.69	0.52	0.65	0.38	.....	.....
3rd day	0.68	0.47	0.64	0.48	0.75	0.32	0.68	0.25
	0.70	0.52	0.78	0.52	0.59	0.31	0.62	0.24
	0.70	0.61	.....	.....	0.67	0.39	.....	.....
4th day	0.71	0.47	0.68	0.43	0.72	0.31	0.70	0.26
	0.65	0.51	0.71	0.45	0.67	0.33	0.65	0.29
	0.69	0.54	0.69	0.37	.....	.....	.....	.....
5th day	0.66	0.43	0.73	0.42	0.77	0.34	0.66	0.30
	0.70	0.45	0.70	0.38	0.74	0.34	0.62	0.29
	0.65	0.56	0.73	0.35	.....	.....	.....	.....

it was noticed that the amount of alkali removed from the soil or diseased parings exceeded the amount of acid removed from them. Because of these findings it was decided to measure the amounts of acid and alkali removed from pea seeds and red kidney bean seeds.

One hundred and ten Alaska pea seeds were soaked for 6 hours in distilled water and then placed in the electrodialysis cell at 80 volts. At the

TABLE VI

ACID AND BASE EQUIVALENTS OF CATHODE AND ANODE SOLUTIONS FROM THE ELECTRODIALYSIS AT 80 VOLTS OF 110 ALASKA PEA SEEDS

INTERVAL	SAMPLE NUMBER	ML. 0.1 N HCl TO NEUTRALIZE CATHODE FLUID	ML. 0.1 N NaOH TO NEUTRALIZE ANODE FLUID
		ml.	*ml.*
0–40 minutes	1	5.95	1.44
	2	5.70	1.23
	3	3.46	1.10
40–80 minutes	1	2.91	1.05
	2	3.48	0.99
	3	3.02	1.05
80–120 minutes	1	2.38	1.05
	2	3.02	1.16
	3	2.92	1.05

TABLE VII

ACID AND BASE EQUIVALENTS OF CATHODE AND ANODE SOLUTIONS FROM THE ELECTRODIALYSIS AT 80 VOLTS OF 55 RED KIDNEY BEANS

INTERVAL	SAMPLE NUMBER	ML. 0.1 N HCl TO NEUTRALIZE CATHODE FLUID	ML. 0.1 N NaOH TO NEUTRALIZE ANODE FLUID
		ml.	*ml.*
0–40 minutes	1	4.80	1.11
	2	5.13	0.95
	3	3.46	0.83
40–80 minutes	1	4.95	1.00
	2	5.95	1.03
	3	4.72	1.11
80–120 minutes	1	4.96	1.28
	2	6.05	1.33
	3	4.48	1.39

end of 40, 80, and 120 minutes the liquids in the end chambers were drawn off, boiled, and titrated with standard sodium hydroxide or hydrochloric acid in order to determine the acid and base equivalents of materials removed from the seeds. The amounts of acid and base removed from Alaska pea seeds are indicated in table VI.

Red kidney beans were electrodialyzed at 80 volts in groups of 55 after being given a 6-hour soaking in distilled water. The amounts of acid and alkali removed from the kidney beans are shown in table VII.

TABLE VIII

ACID AND BASE EQUIVALENTS OF CATHODE AND ANODE SOLUTIONS FROM THE ELECTRODIALYSIS AT 80 VOLTS OF 100 LAXTON'S PROGRESS PEA SEEDS

INTERVAL	SAMPLE NUMBER	ML. 0.1 N HCl TO NEUTRALIZE CATHODE FLUID	ML. 0.1 N NaOH TO NEUTRALIZE ANODE FLUID
		ml.	*ml.*
0–40 minutes	1	8.00	1.83
	2	14.65	3.33
	3	19.10	4.50
	4	12.30	3.08
	5	10.03	1.94
40–80 minutes	1	12.50	3.00
	2	16.05	4.00
	3	17.83	4.90
	4	17.90	4.88
	5	16.10	3.66
80–120 minutes	1	14.70	3.90
	2	15.75	5.00
	3	13.35	4.60
	4	16.30	5.43
	5	15.55	4.13
120–160 minutes	4	8.96	4.66
	5	11.22	4.30
160–200 minutes	4	5.73	3.66
	5	6.54	3.50

Results similar to those obtained with Alaska pea and red kidney bean were obtained with Laxton's Progress pea seeds. These seeds were soaked in lots of 100 for 6 hours and electrodialyzed at 80 volts. Acid and base equivalents of the liquids in the end chambers of the electrodialysis cell after varying periods of treatment are shown in table VIII.

From the tables it can be seen that the bases of the cathode chamber are much more than equivalent to the acids of the anode chamber.

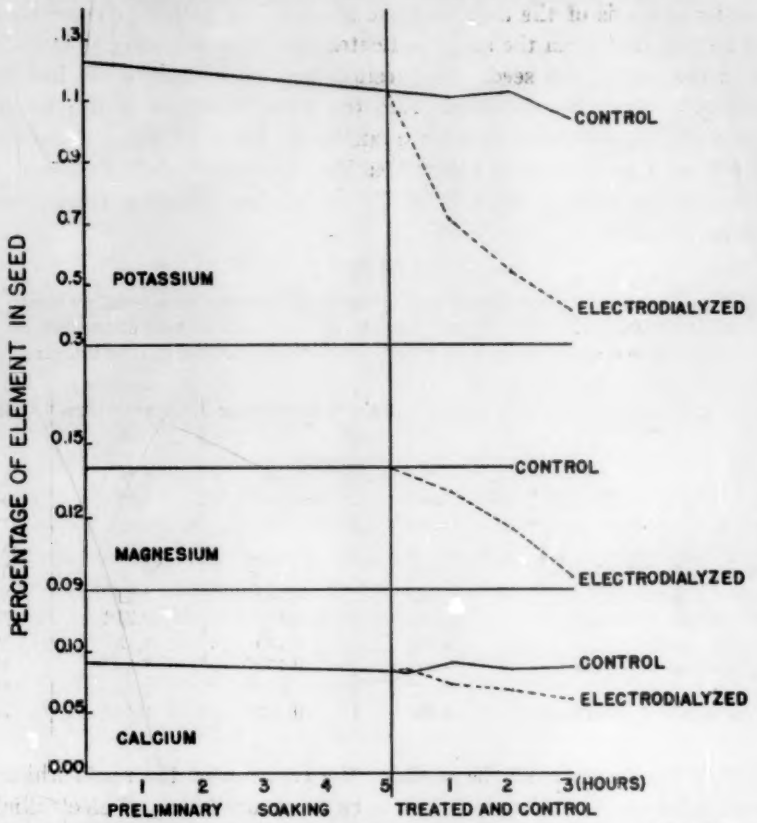


FIG. 2. Percentage of Ca, Mg, and K in seeds after the 5-hour preliminary soaking and after electrodialysis periods varying from 15 minutes to 3 hours.

LOSS OF MINERALS

A study was made of the loss of minerals from seeds during electrodialysis. The seeds used for this part of the work were Laxton's Progress pea seeds. A potential of 150 volts was maintained across the electrodes of the electrodialysis cell. When the current reached 450 milliamperes it was stopped long enough to permit changing of the water in the end chambers of the cell. Seeds were given a 5-hour soaking in distilled water before being placed in the cell. Control lots were given a change of distilled water at the end of the preliminary 5-hour period and were allowed to remain in the

beaker of water as long as the corresponding lot of seeds was in the electrodialysis cell.

At the end of the electrodialysis period both control and treated seeds were placed in a 100° C. oven and dried to be kept for analysis. Methods of analysis have been mentioned earlier.

The calcium, magnesium, and potassium contents of the seeds after different treatments are tabulated in table IX. These elements were determined by analysis of the ash from the seeds. The fact that these elements could be removed from the seeds indicates that they must occur in the ionic form in the soaked pea seed. Magnesium and calcium were not lost to any appreciable extent by diffusion from the control lots of seeds; potassium diffused out in somewhat greater quantities. Each of these elements was removed in much greater quantities by electrodialysis. Calcium, magnesium, and potassium contents of the seeds after different treatments are shown in figure 2.

TABLE IX

COMPOSITION OF PEA SEEDS AFTER HAVING BEEN SUBJECTED TO DIFFERENT TIMES OF ELECTRODIALYSIS. THE AIR DRY WEIGHT OF THE SEED AT THE BEGINNING OF THE EXPERIMENT IS USED AS THE BASIS FOR PERCENTAGE CALCULATIONS

TREATMENT	PERCENTAGE Ca	PERCENTAGE Mg	PERCENTAGE K	SO_4 PRESENT
	%	%	%	
None	0.091	0.140	1.23	Yes
Soaked 5 hours	0.083	.....	.....	Yes
Control 15 minutes	0.083	.....	.....	Yes
Electrodialysis 15 minutes	0.085	.....	.....	Yes
Control 1 hour	0.091	0.140	1.11	Yes
Electrodialysis 1 hour	0.073	0.130	0.712	Yes
Control 2 hours	0.085	0.140	1.13	Yes
Electrodialysis 2 hours	0.067	0.115	0.545	No
Control 3 hours	0.087	.....	1.04	Yes
Electrodialysis 3 hours	0.060	0.095	0.413	No

Either the sulphate of the seeds or the fraction of the seeds which goes to form sulphate on ashing was lost in two or more hours of electrodialysis. Sulphate was not determined quantitatively but its presence was tested for by the use of barium chloride.

ASCORBIC ACID IN THE GERMINATING SEEDS

Ascorbic acid in germinating Alaska pea seeds was determined on the second, third, and fourth days after treatment. These seeds were electrodialyzed for two hours at 80 volts. After treatment, the seeds were kept in flannel rolls moistened with tap water until the time for determination of the ascorbic acid. Amounts of ascorbic acid in the seeds after different lengths of time are shown in table X.

From the results of the analyses presented in table X it can be seen that electrodialysis is followed by low ascorbic acid content of germinating

pea seeds during the early stages of germination. On the fourth day after treatment the ascorbic acid content of the electrodialyzed seeds is almost as high as that of the control lots. This appears significant when it is considered that the control lots have a germination of 90 per cent. while the electrodialyzed seeds have about 35 per cent. germination.

TABLE X

ASCORBIC ACID CONTENT OF ALASKA PEA SEEDS AFTER ELECTRODIALYSIS FOR TWO HOURS AT 80 VOLTS

SAMPLE NUMBER	MG. ASCORBIC ACID PER GRAM OF TISSUE					
	2 DAYS		3 DAYS		4 DAYS	
	CONTROL	ELECTRO-DIALYSIS	CONTROL	ELECTRO-DIALYSIS	CONTROL	ELECTRO-DIALYSIS
1	0.18	0.03	0.25	0.10	0.32	0.26
2	0.16	0.02	0.30	0.11	0.27	0.22

Discussion

The most obvious result of electrodialysis on the seeds used in this work was the reduction of the percentage of germination. By reference to tables II and III it will be noticed that electrodialysis of Alaska pea seeds at 80 volts has a more pronounced effect on their germination than has electrodialysis at 110 volts. No explanation can be offered for this reaction to the electrodialytic process. As electrodialysis takes place at greater rates with increases in voltage, it would appear that the effect on germination must be caused in part by something other than removal of electrolytes from the seeds. It is also difficult to see any close relationship between the removal of calcium, magnesium, and potassium and the percentage germination in the Laxton's Progress pea seeds. Loss of germination is very marked before any great amounts of calcium, magnesium, or potassium are removed from the seeds.

Respiration of Laxton's Progress pea seeds, as measured by their production of carbon dioxide, is greatly affected by electrodialysis at 150 volts. As mentioned before, lowering of carbon dioxide production does not occur to the same extent as germination is lowered for any group of seeds. Respiration seems to bear out the same relation to removal of minerals as does germination to removal of minerals. Carbon dioxide production falls off to a great extent before any appreciable loss of magnesium or calcium takes place. Potassium, however, is lost early in the electrodialysis process and may possibly be associated with decrease in respiratory activity. Although carbon dioxide production is less following electrodialysis, it is interesting to note that seeds which fail to germinate because of the treatment still give off carbon dioxide.

Calcium, magnesium, and potassium are all lost to varying extents from Laxton's Progress pea seeds during the course of electrodialysis. Although

some potassium was lost from the control lots of these seeds while soaking in distilled water, no appreciable amounts of calcium and magnesium were lost from the control lots. The difficulty found in the ashing of seeds electrodialyzed for an hour or more may have been caused by a lesser amount of mineral matter in the seeds. A phenomenon similar to this is encountered in the study of burning qualities of tobacco. HALEY and OLSON (**2**) suggest that good combustion of cigar-leaf tobacco is associated with potassium compounds which give rise to potassium carbonate during burning, and that the presence of such compounds may serve to intensify the catalytic effects of iron in this process. It has also been reported by HALEY, NASSET, and OLSON (**1**) that in practically all cases there is a parallelism between the burning qualities of tobacco and the water-soluble alkalinity of the ash.

It is demonstrated that a greater quantity of base than acid is removed from the seeds during electrodialysis. This is shown by the amount of acid necessary to neutralize the cathode chamber, being much more than equivalent to the amount of alkali necessary to neutralize the anode chamber after dialysis. Possibly this may give some indication of the state of cations in protoplasm. From this it would appear that the cations are either adsorbed on surfaces or are in combination with anions which cannot penetrate the membranes through which they would have to pass in order to reach the anode chamber. When adsorbed cations are removed from soils by electrodialysis, they are replaced by hydrogen ions from the water in which electrodialysis takes place. It is very probable that a similar reaction takes place in seeds which lose cations through electrodialysis.

The effect of electrodialysis on the ascorbic acid content of Alaska pea seeds electrodialyzed for two hours at 80 volts is very marked. During the early stages of germination this treatment was followed by very low amounts of ascorbic acid in the seeds. By the end of the fourth day after treatment, however, the electrodialyzed seeds contained almost as much ascorbic acid as the control lots. This seems significant in view of the fact that germination in the treated seeds was much lower than in the control lots.

Summary

1. Germination of Laxton's Progress and Alaska pea seeds, and red kidney beans was markedly decreased by electrodialysis of the seeds.

2. Longer periods of electrodialysis were accompanied with lesser percentages of germination.

3. Production of carbon dioxide was studied in Laxton's Progress pea seeds after they had been electrodialyzed, and it was found that this treatment had a great inhibiting effect on subsequent carbon dioxide production.

4. Calcium, magnesium, and potassium were all lost at a much greater rate from electrodialyzed Laxton's Progress pea seeds than from control lots.

5. Cations removed from all the lots of seeds studied were more than equivalent to the removed anions.

6. In the ash of Laxton's Progress pea seeds electrodialyzed two hours or more at 150 volts there was no sulphate ion present.

7. Electrodialysis of Alaska peas for two hours at 80 volts was followed by a very low ascorbic acid content during the early stages of germination. By the end of the fourth day the electrodialyzed seeds contained nearly as much ascorbic acid as the controls.

This work was done partly in the Department of Horticulture of the Pennsylvania State College, and the remainder in the Department of Botany of the University of Chicago. The writer wishes to express his thanks to Dr. C. A. SHULL, Dr. W. B. MACK, and Dr. E. C. DUNKLE for help rendered during the course of the work. Dr. CHARLES L. HAMNER did the major portion of the work in the ascorbic acid determinations.

THE UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

LITERATURE CITED

1. HALEY, D. E., NASSET, E. S., and OLSON, OTTO. A study of certain constituents of the leaf and their relation to the burning qualities of tobacco. Plant Physiol. **3**: 185–197. 1928.
2. ————————, and OLSON, OTTO. On the relation of potassium to iron in the combustion of cigar-leaf tobacco. Science n.s. **70**: 17–18. 1929.
3. LISBONNE, M., and VULQUIN, E. Inactivation de l'amylase du malt par la dialyse électrique. Activation par les électrolytes. Compt. Rend. Soc. Biol. **72**: 936–938. 1912.
4. ————————, and ————————. La dialyse électrique des diastases. Application du principe a la purification du malt. Jour. Physiol. et Path. gen. **15**: 23–29. 1913.
5. MACLINN, WALTER A., and FELLERS, CARL R. Ascorbic acid in tomatoes and tomato products. Massachusetts Agr. Exp. Sta. Bull. 354. 1938.
6. MAIGNON, F. Action de l'électrolyse sur l'activité des diastases. Compt. Rend. Soc. Biol. **93**: 400–402. 1925.
7. MAKSIMOW, A. Electrodialysis in plants. Acta Soc. Botan. Polon. **13**: 231–250; Uprawa Roslin i Nawozenie **8**: 58–60. 1938.
8. MATTSON, SANTE. Electrodialysis of the colloidal soil material and the exchangeable bases. Jour. Agr. Res. **33**: 553–567. 1926.
9. MENAKER, M. H., and GUERRANT, N. B. Standardization of 2,6 dichlorophenolindophenol. Ind. & Eng. Chem. Anal. Ed. **10**: 25–26. 1938.
10. MOORE, JAMES C., REEVES, R. G., and HIXON, R. M. Electrodialysis as a means of studying biochemical differences in abnormal apple tissue. Plant Physiol. **2**: 313–324. 1927.
11. MULLISON, WENDELL R. Electrodialysis of pea seeds. Plant Physiol. **14**: 583–587. 1939.

12. Scott, W. W. Standard methods of chemical analysis. Van Nostrand, New York. 1925.
13. Seifriz, W. Breakdown of fruit and vegetable tissue due to an electric current. Plant Physiol. **11**: 195–200. 1936.
14. Skinner, W. W. Editor, Official and tentative methods of analysis of the Association of Official Agricultural Chemists. Washington, D. C. 1940.

EFFECTS OF OXYGEN TENSION ON CERTAIN PHYSIOLOGICAL RESPONSES OF RICE, BARLEY, AND TOMATO

J. VLAMIS AND A. R. DAVIS

(WITH NINE FIGURES)

The relation of oxygen pressure to some metabolic processes of plants has been examined in recent experimental work with particular reference to the energy requirements of active cells. The reactions studied under the most carefully controlled conditions are accumulation of salt and respiration by excised roots and storage organs. This is particularly true of the work by HOAGLAND and his associates at the University of California, and STEWARD at the University of London.

Other investigations have been concerned with the response of entire plants to oxygen deficiency in the roots. Earlier studies compared plants grown in submerged soils with those grown in well-drained soils. It is evident that the control over such experiments is complicated by the influence of several variables such as carbon dioxide pressure, accumulation of anaerobic products, and the intervention of microbiological activity. Greater control has been possible when water cultures have been employed, exposing the root systems to continuous streams of air or relatively pure nitrogen. The influence of oxygen supply on tomato, buckwheat, barley, lupine, and other plants has been studied in this way.

The oxygen relations of the rice plant present a particularly interesting and important problem since as a crop it is grown under conditions (apparently anaerobic) which inhibit the growth of many field plants. This ability of rice to thrive in submerged soil has been observed and confirmed by some research workers and denied by others. A rather large number of diverse experiments on a qualitative scale has left suspended a final estimation of the capacity of rice, real or apparent, to grow in an anaerobic root environment.

This investigation has proceeded in several directions with the hope of providing additional information on the above problem. The response of rice roots to different oxygen tensions has been studied, especially with respect to respiration and mineral absorption. Barley and tomato were employed as comparison plants; the former because of its non-critical oxygen requirement, and the latter on account of its high need for this element. As shown generally in many greenhouse experiments, barley may be considered as occupying an intermediate position between rice and tomato in this respect.

These experiments are presented in three parts. The first concerns the salt accumulation and respiration of excised roots under different oxygen pressures, and the accumulation of salt by roots of entire plants under

aerobic and anaerobic root exposure. The second covers the growth and salt accumulation by entire plants in drained and submerged soil. The third part deals with the growth and salt accumulation by plants in culture solutions exposed to streaming air, nitrogen, carbon dioxide, and methane.

Methods and results

Accumulation of salt and respiration by excised and intact roots

Accumulation of salt by excised roots.—The use of salt accumulation and respiration as an index of metabolic response to oxygen pressure was employed by Steward (18) using potato discs under controlled conditions. He found a great increase in the accumulation of bromide and in respiration, measured by carbon dioxide production, as the oxygen content was raised from low partial pressures up to twenty per cent. Above this value and up to pure oxygen there was little increase in salt accumulation or respiration. In subsequent work Steward and his coworkers (19) obtained the same type of curve for carrot and artichoke storage tissue. On the other hand, excised potato roots showed a critical value in oxygen limitation at ten per cent. This is approximately the curve described by Hoagland and Broyer (13) on excised barley roots for bromide, potassium, and nitrate accumulation, sugar loss, and carbon dioxide production.

Before proceeding to a comparison of the behavior of excised rice, barley, and tomato roots under different oxygen pressures, several preliminary experiments were conducted on barley roots to clarify some interesting questions raised by previous workers. Hoagland and Broyer showed the importance of the salt and sugar content of tissues involved in salt accumulation over short time intervals. The tissues used in their oxygen-variable experiment were of the low salt-high sugar type.

The influence of salt status on the response of excised barley roots to various oxygen pressures was first examined. The technique with minor elaborations is that described elsewhere (13). Seeds were germinated on wire screens between cheese-cloth and after a week placed in paraffined corks. Twenty-four such corks were placed in 4-liter enamel pans containing Hoagland's solution. The high-salt plants received full strength Hoagland's solution every five days, the low-salt plants were given one-quarter concentration of this solution every two weeks.

After four weeks the roots were excised and placed in 2-liter jars containing 0.005 M KBr for twenty-four hours; each jar was aerated with a different mixture of oxygen and nitrogen ranging from 0.2 to 100 per cent. oxygen. At the end of the absorption period the roots were centrifuged, frozen at 0° F., then thawed and the sap expressed for analysis. The determination of oxygen in the gas mixtures was by the Winkler method (21) for the range 1 to 100 per cent., and by a colorimetric method (9) based on oxidation of metallic copper to blue cupric ion, for the range 0.02 to 2 per cent.

Analysis of the expressed sap of low-salt and high-salt roots showed that salt status affected the absolute level of bromide accumulation without influencing the shape of the curve. That is, the inflection of the curve for both types of roots occurred at the same partial pressure of oxygen, about 3 per cent.

The effect of time upon the rate of bromide absorption was also examined. Intervals of 1, 4, 12, 24, 36, and 48 hours magnitude for each oxygen pressure disclosed the steepest gradient within 12 hours exposure, with only slight increases thereafter. For this reason the 24- and 36-hour periods were chosen as suitable intervals.

Experiment 1. Comparison of rice, barley, and tomato.—By the procedure outlined above excised roots of rice, barley, and tomato were obtained.

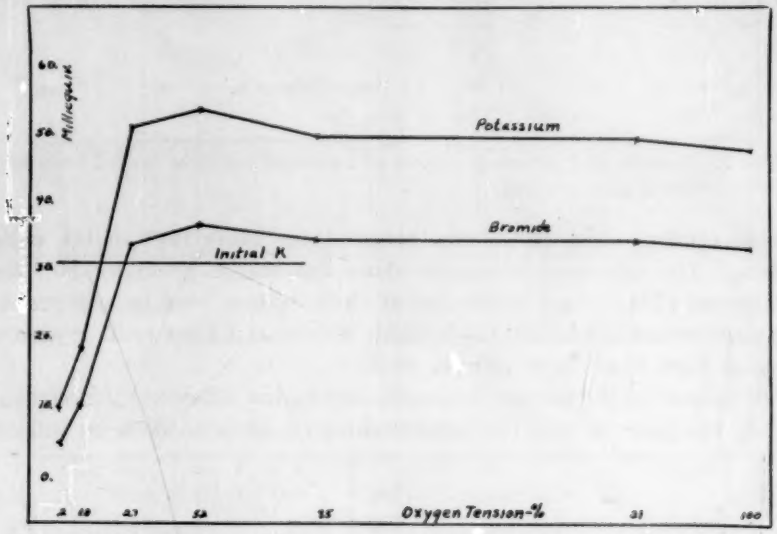


FIG. 1. Bromide and potassium content of expressed sap from excised roots of barley 24 hours in 0.005 M KBr solution.

Three varieties of rice were employed; Caloro variety from California, Hambas lowland, and Carreon upland varieties from the Philippines. Sacramento barley and Santa Clara tomato were the varieties of these plants used.

The roots were exposed to 0.005 M KBr and aerated for 24 hours with oxygen-nitrogen mixtures covering the range mentioned above. The original salt content was not uniform for the three sets as shown by the differing initial potassium concentrations at the time of excision. This accounts for the different maxima of bromide accumulation obtained with each of the root systems (figs. 1, 2, 3).

The bromide accumulation curve of all three sets of roots has the same general shape with the greatest rate of bromide absorption up to 3 per cent. oxygen and very slight increase at higher partial pressures of oxygen. It is interesting to note that as the oxygen pressure falls below 1 per cent. the

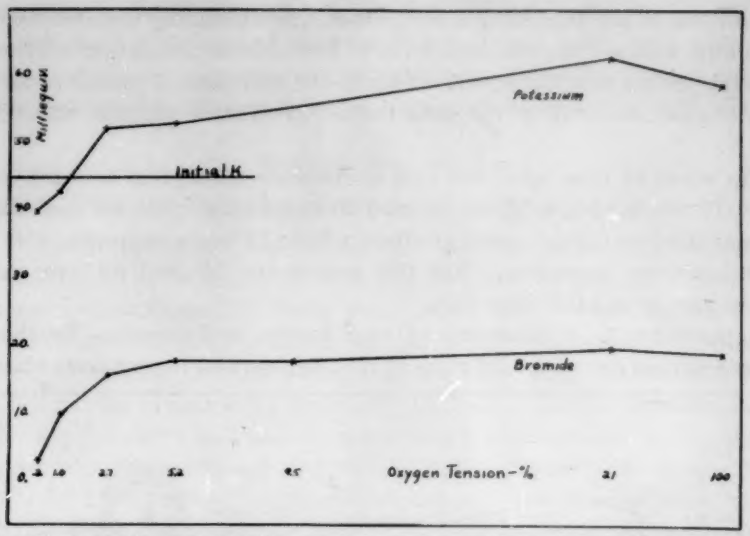


FIG. 2. Bromide and potassium content of expressed sap from excised roots of rice 24 hours in 0.005 M KBr solution.

bromide concentration of the sap fails to rise above that in the culture medium. The curves approximate those for barley given by HOAGLAND and BROYER (13). An examination of their data as read from a graph in their publication, shows that the bromide uptake at 4.1 per cent. oxygen was almost as high as at 21 or 100 per cent.

For potassium the picture is complicated by the different initial amounts present. In spite of this, the potassium curves likewise show an inflection

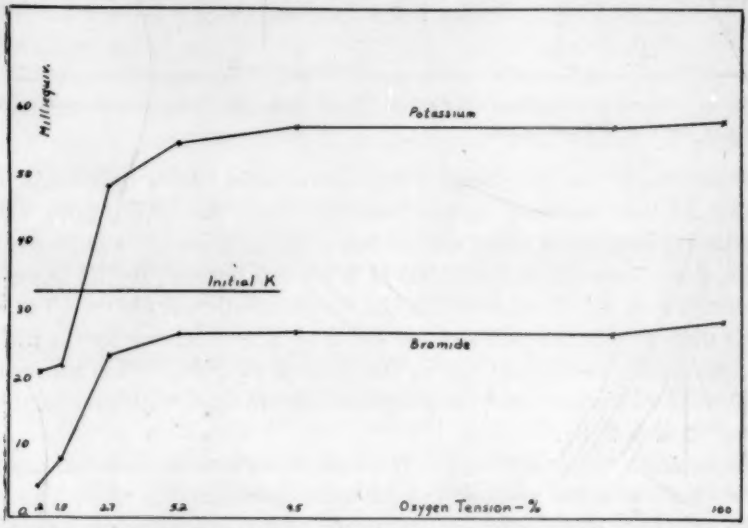


FIG. 3. Bromide and potassium content of expressed sap from excised roots of tomato 24 hours in 0.005 M KBr solution.

at 3 per cent. oxygen. Below this pressure there is a loss of potassium from all roots, a great leakage from barley and tomato, but very slight from rice. The significance of this remains to be determined.

Experiment 2. Accumulation by intact roots.—In the absence of any

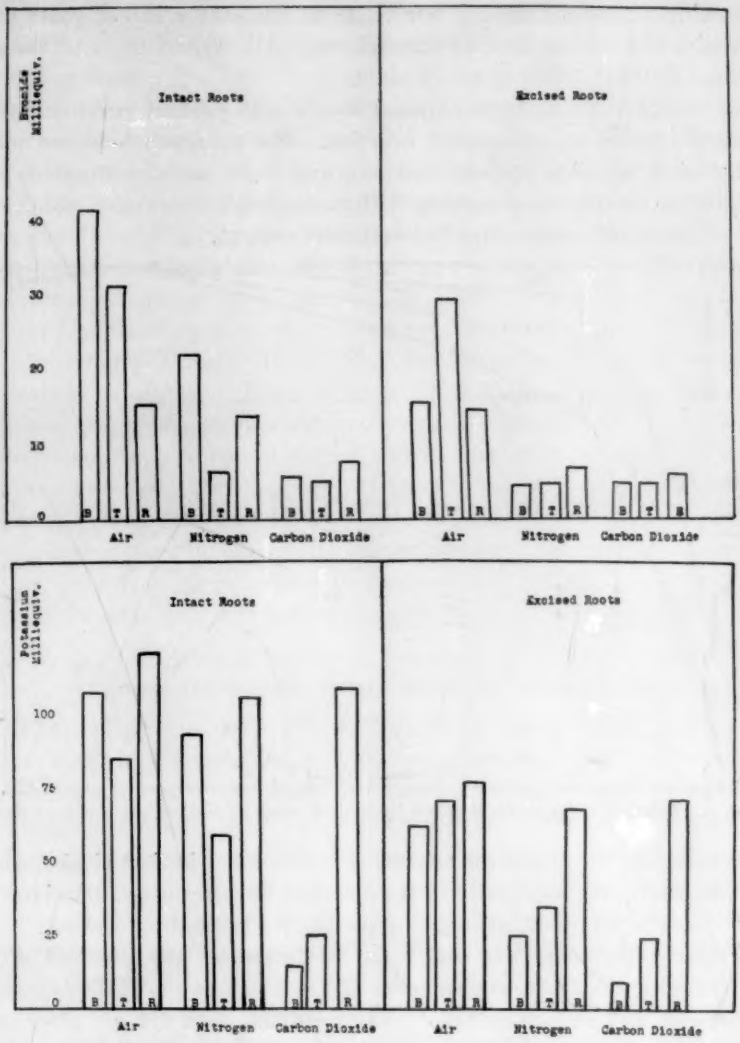


FIG. 4. Bromide and potassium content of excised and intact roots 36 hours in 0.005 M KBr solution. Barley, B; tomato, T; rice, R.

decisive differences in oxygen sensitivity by excised roots of the three plants under investigation, tests were undertaken to determine the influence of the shoot on the behavior of roots exposed to air or nitrogen.

Root systems of entire plants were placed for 36 hours in 2-liter jars containing 0.005 M KBr solutions aerated with nitrogen, air, and carbon

dioxide. The nitrogen contained 0.2 per cent. oxygen, and the carbon dioxide about the same. Excised roots from similar plants were included in the solutions along with the intact roots. The results are shown in figure 4.

Bromide accumulation did not occur in excised or intact roots of any plants with the carbon dioxide atmosphere. All excised roots of the nitrogen series likewise failed to accumulate.

The roots of entire plants exposed to nitrogen showed great differences. The tomato failed to accumulate bromide. Barley roots, however, showed a fairly high bromide content and rice relatively more, compared to the respective accumulation occurring with the aerated treatment, which serves as the standard of comparison for each root system.

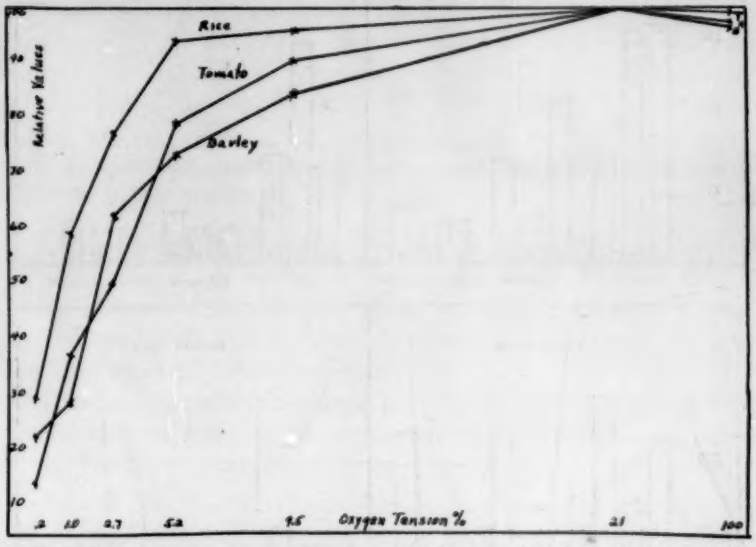


FIG. 5. Relative oxygen consumption by excised roots of barley, tomato, and rice.

The relations for potassium are not as uniform but indicate leakage under anaerobic conditions, especially in the presence of pure carbon dioxide. The aerated roots of all plants again show the highest potassium content.

Another experiment was conducted to determine the effect of a 7-day treatment with the three atmospheres of gas prior to the KBr exposure to air, nitrogen, and carbon dioxide. The resulting data did not alter in any material way the relationships indicated in the preceding paragraphs.

Experiment 3. Respiration of excised roots.—The inter-connection of accumulation and respiration (carbon dioxide production) has been demonstrated by STEWARD (**18**), and by HOAGLAND and BROYER (**13**). In their studies these responses were measured simultaneously and showed an approximately parallel sensitivity to oxygen tension. In the present case this procedure was difficult to follow and it was necessary to make separate measurements of respiration as oxygen consumption.

The respiratory behavior of excised roots from plants grown aerobically in solution was followed with the FENN apparatus (**7**). The oxygen uptake was determined over short time intervals until an equilibrium rate was established. This was achieved within one hour for each partial pressure of oxygen. The data are plotted in figure 5 with values at 21 per cent. oxygen taken as 100. While some differences appear in the curves, they are not significant.

The preceding experiments measured the effects of several partial pressures of oxygen on excised roots and the roots of whole plants previously grown in the usual unaerated culture solutions. The excised roots of the three plants behaved similarly in different oxygen pressures as measured by bromide content and oxygen consumption. On the other hand, the root behavior of whole plants showed the extreme dependence of the tomato on root aeration, as compared to the relative indifference of rice and barley. The latter two plants apparently derive a considerable portion of root oxygen by transporting it through the shoot. This is indicated by the ability of these roots with attached shoots to accumulate under anaerobic root conditions, when an aerobic atmosphere is provided to the shoots.

These tests were all conducted over short absorption periods. Furthermore, they have no direct bearing on growth but only on respiration and salt uptake. The continued effect of several types of root aeration on plant growth was therefore investigated. Experiments were undertaken to determine the growth of these plants in drained and submerged soils, and in culture solutions exposed to different gases for a major part of the vegetative cycle.

GROWTH OF PLANTS IN DRAINED AND SUBMERGED SOIL

Recognition of the importance of oxygen supply of roots in the growth of plants is shown by many investigations utilizing soil and water cultures. The inability of many crop plants to tolerate root submergence when grown in soil is usually attributed to oxygen deficiency. Several other important considerations have been neglected, however, especially the increased concentration of carbon dioxide and the products of fermentation; both inevitable corollaries to anaerobiosis characteristic of water logged or heavy soils.

The literature is comprehensively surveyed in a recent paper on oxygen deficiency in soils (**5**). Only the more pertinent references are reported here. BERGMAN (**2**) obtained no deleterious effects due to submergence of potted *Ranunculus abortivus, R. sceleratus,* and *Cyperus alternifolius,* whereas *Impatiens balsaminea* succumbed to such treatment. He suggests the presence of aerenchyma in the first group of plants. CANNON (**3**), in an extensive study of a large number of species, concluded that plants possess different sensitivity to low oxygen tension; but his results were indecisive, possibly due to inexact control of conditions and inadequate sampling. WEAVER and HIMMEL (**22**) found that submergence improved the growth of certain hydrophytes.

HARRISON and AIYER (**11**) analyzed the gases of swamp rice soils and discovered large amounts of methane with some nitrogen, carbon dioxide, and hydrogen. Further studies (**12**) convinced them of the importance of surface algae as sources of molecular oxygen for the roots of the submerged rice plant. The data on which they base this conclusion, however, are subject to a contrary interpretation in the presence of a large experimental error. SETHI (**16**) as a result of pot and field experiments identified two types of roots in rice, one resembling the structure in wheat and the other containing air spaces characteristic of aquatic roots. He obtained superior shoot and root growth under waterlogged conditions but the fruit yield was

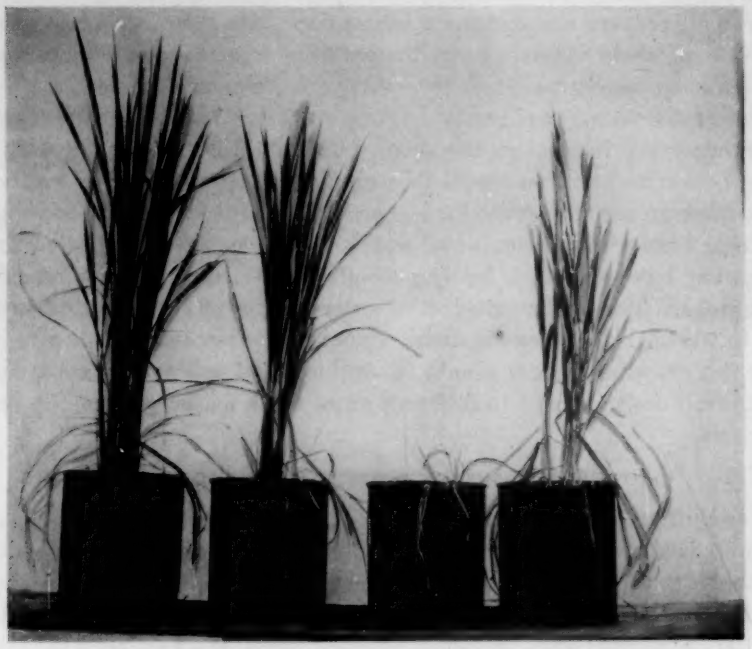


FIG. 6. Plants grown for six weeks in clay soil, drained and submerged. Left to right: rice submerged, rice drained, barley submerged, and barley drained.

only equal to the control. CONWAY (**5**) using *Cladium mariscus* showed a high oxygen content in intact roots, while excision resulted in a very low oxygen value. VAN RAALTE (**15**) found a similar situation to exist in the roots of rice.

Experiment 4.—The growth of most plants in soil, potted or in the field, is predicated on adequate drainage to maintain an aerobic environment around the roots. Rice forms a notable exception to this in agriculture, and is grown almost universally under a standing water depth varying from a few inches to several feet.

A comparison of the growth of rice (3 varieties), barley, and tomato was made under similar conditions of soil drainage and submergence in a

first effort to isolate the specific factors responsible for that behavior. Seeds were germinated by the method referred to previously; after growing to a length of about 15 cm., they were transferred carefully to gallon cans filled with loam or clay soil. One-half the number of cans were drained by means of numerous perforations. Protection of the plants from direct radiation was given for 48 hours to allow recovery from transplanting. All plants retained their apparent vigor during this period.

The submerged plants were gradually exposed to increasing soil moisture until one inch of free water stood above the soil surface and was kept at that level. The drained set received additions of water daily. At the end of

FIG. 7. Tomato plants grown for six weeks in clay and loam soil, drained and submerged. From left to right: loam submerged, loam drained, clay submerged, and clay drained.

six weeks the soil was easily washed away from the roots, photographs taken (figs. 6, 7), fresh weights measured, and after freezing the tissues the sap was expressed and analyzed for potassium. The results are given in table I.

Rice consistently excelled under submerged conditions, particularly with respect to the root system; barley and tomato failed to develop at all in the submerged clay, and grew only slightly in the submerged loam.

The potassium content of the sap of rice shoots was about equal under both growth conditions, but the drained roots of rice averaged twice the potassium concentration of the submerged roots. This difference may be due in a large measure to the dilution effect exercised by the excess water on

the salt concentration of the flooded soil. No measurable sap could be extracted from submerged barley and tomato.

The improved growth of rice in a saturated soil provides a sharp contrast to the need of drainage exhibited by barley and in particular the tomato. This difference is much greater than that obtained in the preceding experiment with aerobically grown plants subsequently exposed to anaerobic KBr solutions for short periods of time. From both these experiments it appears that the advantage of intact over excised roots is derived from the presence of a shoot system having access to an aerobic atmosphere. This advantage is greatest in the rice plant, much less in barley, and negligible in tomato; it suggests the quantity of oxygen which may be transported from the shoots to the roots by anatomical adaptation. The evidence further

TABLE I

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF PLANTS GROWN 6 WEEKS IN DRAINED AND SUBMERGED CLAY AND LOAM

SOIL	PLANT	SOIL TREATMENT	SHOOTS		ROOTS	
			FRESH WEIGHT	K CONC.	FRESH WEIGHT	K CONC.
			gm.	*m.eq.*	*gm.*	*m.eq.*
Clay	Barley	Drained	18.0	167	2.2	57
		Submerged	2.0	.....	0.2	.....
	Tomato	Drained	12.2	.....	1.2	.....
		Submerged	2.7	.....	1.4	.....
	Upland rice	Drained	25.7	171	11.1	65
		Submerged	50.5	162	50.0	35
	Lowland rice	Drained	20.0	196	9.2	55
		Submerged	34.5	209	38.6	32
	Caloro rice	Drained	20.6	167	6.3	71
		Submerged	34.8	159	27.5	37
Loam	Barley	Drained	35.1	246	13.4	32
		Submerged	18.5	125	8.0	18
	Tomato	Drained	55.5	.....	9.4	.....
		Submerged	9.1	.....	4.5	.....
	Caloro rice	Drained	44.7	187	17.5	15
		Submerged	58.8	208	27.6	11

suggests this as the most reasonable explanation of the activity of intact and excised roots. Of course, this in no way suffices to explain the increase in rice growth as a consequence of soil submergence.

An important distinction between drained and flooded soil arises from the relative availability of oxygen which affects the plant directly; and indirectly by its influence on the activity of soil bacteria and on the chemical constitution of the soil solution. This difference may be expressed by a number of interacting variables of which the more important are oxygen deficiency, carbon dioxide accumulation, products of fermentation, and the presence of reduced ions.

An improved control of these factors was attained by the use of water cultures through which several gases were passed separately.

GROWTH IN WATER CULTURE

The literature on oxygen relations in water culture media is extensive and a recent survey of it is given by DURELL (**6**). HALL, BRENCHLEY, and UNDERWOOD (**10**) noticed a 50 per cent. increase in total dry weight of lupine and barley upon aerating the nutrient solution. FREE (**8**) found buckwheat indifferent to air, oxygen, or nitrogen gases forced through a culture solution, whereas carbon dioxide proved lethal after a short exposure. CLARK and SHIVE (**4**) showed that aeration gave large increases in both shoots and roots of tomato.

ARRINGTON and SHIVE (**1**) doubled the yield of tomato by aerating, and found some carbon dioxide accumulation in the solution to be without effect.

TABLE II

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF PLANTS GROWN 6 WEEKS IN CULTURE SOLUTIONS WITH GAS EXPOSURE

PLANT	ROOT TREATMENT	SHOOTS	ROOTS	
		FRESH WEIGHT	FRESH WEIGHT	K CONC.
		gm.	*gm.*	*m.eq.*
Barley	Control	24.0	10.9	101
	Air	24.6	13.5	99
	Nitrogen	13.2	7.9	102
	Carbon dioxide	1.5	2.7	.....
Tomato	Control	49.2	15.1	88
	Air	120.0	34.5	94
	Nitrogen	7.1	1.9	.....
	Carbon dioxide	3.5	1.2	.....
Upland rice	Control	38.1	13.7	82
	Air	34.6	14.9	86
	Nitrogen	37.2	13.8	81
	Carbon dioxide	3.1	1.0	.....

DURELL (**6**) likewise obtained a distinct increase in fruit and vegetative growth of tomato under aerated conditions. STILES and JØRGENSEN (**20**) improved barley and balsam growth by aeration, while buckwheat was unaffected.

Recently SHIVE (**17**) discovered different oxygen requirements for soybean, oat, and tomato as reflected by yield. Absorption of nitrate ions over a short interval was high at low oxygen pressures and decreased at higher pressures, and the absorption of ammonium nitrogen varied inversely as the nitrate.

The effect of root exposure to various gases passed rapidly through the culture solution was studied. The medium consisted of HOAGLAND'S solution and the plants were grown in 2-quart jars. Air, carbon dioxide, and nitrogen (0.2 per cent. oxygen as impurity) were used separately in addition to the unaerated control. The rate of aeration was maintained at 3 liters per hour and the gases forced through sintered glass aerators for dispersion.

Experiment 5.—Seedlings were started aerobically and when the shoots reached about 10 cm. in length were set out in the jars on paraffined corks; seven seedlings of barley and rice per jar and one of tomato. The number of individuals used was small, but due to technical obstacles it was preferred to repeat experiments entirely rather than to use larger populations in a more limited number of tests. The extreme and consistent differences that occurred also tend to reduce the difficulties inherent in variability or imperfect control of conditions.

The results shown in table II support most of the observations presented from the soil experiments. The unaerated control is taken as the standard of comparison. Aeration increased tomato shoot and root growth almost

FIG. 8. Barley and tomato plants grown for six weeks in culture solutions with various gas exposures to the root system. From left to right in both series: control (unaerated), nitrogen, carbon dioxide, and air.

equally by over 100 per cent. in fresh weight. Barley consistently gave a small increase in shoot and root growth. The effect of aeration on rice fresh weight was negligible, the experimental error being larger than the treatment variations of any one experiment.

Exposing the roots to a nitrogen atmosphere reduced tomato shoots and roots by about 90 per cent. Barley shoots were 45 per cent. and the roots 30 per cent. smaller than the control. Rice root and shoot growth were of the same order of magnitude as the control. The plants at the time of harvesting are shown in figures 8 and 9.

Carbon dioxide was immediately lethal to all plants. This can hardly be purely the influence of oxygen limitation in view of the foregoing evidence; it more likely represents a specific lethal action of the carbon dioxide

superimposed on oxygen deficiency. Other experiments with barley have indicated that a 20 to 30 per cent. partial pressure of carbon dioxide is toxic even if the entire residual pressure of 70 to 80 per cent. consists of oxygen.

Analyses of expressed sap were impossible for those treatments which yielded insufficient material. The sap analysis of shoots was also omitted in the face of evidence of great uniformity in virtually any treatment yielding a measurable amount of growth. Where possible the sap of roots alone was analyzed for potassium.

The tomato root sap had a high concentration of potassium in the control and that of aerated roots was slightly higher. The difference between

FIG. 9. Rice plants grown for six weeks in culture solutions with various gas exposures to the root system. Left to right: control (unaerated), nitrogen, carbon dioxide, and air.

the two, however, is trivial compared to that expressed by fresh weight. Barley roots gave no significant difference in potassium whereas fresh weight differences did occur. This indicates that whatever restricted growth is made by barley and tomato roots under adverse oxygen conditions, the character of that growth proceeds without impairment of the salt accumulating mechanism in those particular tissues. Rice roots likewise showed uniform potassium concentrations, which are in accordance with the uniformity of fresh weight values for this plant.

Inasmuch as the nitrogen contained a small amount of oxygen (0.2 per cent.), an experiment was designed to reduce this impurity as much as pos-

TABLE III

COMPOSITION OF METHANE GAS

CONSTITUENT	PERCENTAGE
Methane	85.0
Ethane	8.7
Propane	5.0
Butane and higher hydrocarbons	1.3
Oxygen (less than 0.02 per cent.)	
Total	100.0

sible. For this purpose commercial methane gas was selected, the gas having the composition given in table III, with no carbon dioxide or monoxide present, or oxygen within the limits of sensitivity of the method used.

Experiment 6.—The procedure followed was similar to the preceding one substituting methane for nitrogen. The plants were grown out of doors instead of in the greenhouse to avoid any danger from the methane. The experiment continued for six weeks.

The data appear in table IV giving fresh weight of shoots and roots, and the potassium concentration of expressed root sap. Aeration improved tomato considerably, and barley slightly; it exerted no influence over rice, however, except for a discoloration at the root tips indicating some form of injury. This apparent damage to the rice root has been observed in several subsequent experiments.

Methane gas caused an absolute cessation of tomato growth immediately on exposure. Barley was reduced by about 60 per cent., a greater loss than

TABLE IV

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF PLANTS GROWN OUTDOORS 6 WEEKS IN CULTURE SOLUTIONS WITH GAS EXPOSURE

PLANT	ROOT TREATMENT	SHOOTS	ROOTS	
		FRESH WEIGHT	FRESH WEIGHT	K CONC.
		gm.	*gm.*	*m.eq.*
Barley	Control	49.2	22.6	103
	Air	53.5	25.3	98
	Methane	20.2	10.2	58
Tomato	Control	41.8	17.4	102
	Air	64.5	31.0	101
	Methane	3.2	1.2	—
Upland rice	Control	25.5	11.1	100
	Air	25.0	15.3	101
	Methane	24.0	24.7	97
Lowland rice	Control	33.6	19.3	91
	Air	31.2	15.4	88
	Methane	33.7	29.2	90

in the presence of nitrogen. The methane treatment resulted in a fresh weight increase of rice roots amounting to about 70 per cent. over the control. The nature of this stimulation of rice from methane exposure has not been determined, but may have the same origin as the beneficial effect of soil submergence on this plant.

The potassium content of the roots assumed the general proportions obtained in the preceding experiment with one exception. The barley roots exposed to methane showed a severe reduction in potassium content which was almost proportional to the decrease in fresh weight.

The apparent stimulation of rice roots by methane offers abundant room for speculation. The use of this gas was suggested by its frequent and abundant occurrence in swamp rice fields (**14**). For the purpose of these experiments it had one advantage over nitrogen in its lack of oxygen. From the effect of methane on barley its behavior would seem to be that of any

TABLE V

COMPOSITION OF NITRATE, AMMONIUM, AND AMMONIUM NITRATE SOLUTIONS

NITRATE		AMMONIUM		AMMONIUM NITRATE	
SALT	CONC.	SALT	CONC.	SALT	CONC.
	M		*M*		*M*
$Ca(NO_3)_2$	0.005	NH_4Cl	0.015	NH_4NO_3	0.015
KNO_3	0.005	KCl	0.005	KCl	0.005
$MgSO_4$	0.002	$MgSO_4$	0.002	$MgSO_4$	0.002
KH_2PO_4	0.001	KH_2PO_4	0.001	KH_2PO_4	0.001
NaCl	0.015	$CaCl_2$	0.005	$CaCl_2$	0.005
Fe	Trace	Fe	Trace	Fe	Trace

Micro-elements added as Hoagland's supplementary A–Z solution.

inert oxygen-free gas. The present data indicate that it inhibits the growth of barley roots to only a slightly greater extent than nitrogen with a 0.2 per cent. oxygen impurity, although the effect on potassium content was more drastic. It remains to be seen whether methane has a specific stimulating influence on rice, or if its superiority rests on purely anaerobic qualities. The rough similarity between rice grown in submerged soil and in water culture exposed to methane offers the prospect of further elucidation of the anaerobic preference exhibited by entire rice plants.

As the culture solution used contained considerable amounts of nitrate, the possible rôle of this anion as a source of respiratory oxygen was examined. It has been shown with soybean plants (**17**) and excised barley roots (**13**) that nitrate is absorbed in large quantities and reduced by the root cells. This creates the possibility that in an anaerobic environment roots may absorb large amounts of nitrate, while maintaining relatively low internal concentrations of this ion by subsequent reduction. The oxygen so released presumably would be utilized in oxidative processes. A test was designed to provide salt nutrients of nitrogen in three forms: nitrate, am-

monium, and ammonium nitrate in the presence of air and methane forced through the root medium with the shoots exposed to the air.

Experiment 7.—Plants of barley and rice were grown in unaerated 2-quart jars containing quarter strength HOAGLAND'S solution for three weeks and then transferred to the experimental conditions. The composition of the three solutions containing nitrate, ammonium, and ammonium nitrate, respectively, is given in table V. The osmotic value of the solutions was the same and the nitrogen concentration was also equal for each form of ion. Each solution was treated with the usual aeration and with methane, requiring outdoor growth as before. After two weeks the plants were harvested and the sap of roots expressed and analyzed for potassium. The results are presented in table VI and they do not assign any importance to

TABLE VI

FRESH WEIGHT AND POTASSIUM CONCENTRATION OF EXPRESSED SAP OF BARLEY AND RICE PLANTS GROWN OUTDOORS FOR 2 WEEKS

PLANT	SOLUTION	ROOT TREATMENT	SHOOTS	ROOTS	
			FRESH WEIGHT	FRESH WEIGHT	K CONC.
			gm.	*gm.*	*m.eq.*
Barley	Nitrate	Air	24.5	15.1	85
		Methane	17.0	9.4	70
	Ammonium	Air	21.9	13.3	95
		Methane	14.0	8.6	79
	Ammonium nitrate	Air	27.0	12.2	99
		Methane	18.6	6.4	72
Upland rice	Nitrate	Air	24.0	12.9	114
		Methane	23.1	14.6	104
	Ammonium	Air	22.9	12.1	100
		Methane	23.8	13.7	122
	Ammonium nitrate	Air	23.6	12.7	93
		Methane	21.4	14.2	105

nitrate as a source of oxygen for the growth of rice or barley under anaerobic root conditions.

The fresh weight values of barley indicate a fairly uniform inhibition due to the exclusion of molecular oxygen from the roots regardless of the form of nutrient nitrogen employed. Rice likewise fails to derive any benefit from nitrate over other sources of nitrogen when an anaerobic atmosphere prevails in the root system.

With regard to the potassium content of the expressed sap, in the case of barley the aerated roots all have slightly higher values than methane-treated roots of corresponding solutions. For rice the effect of aeration was not noticeably different from that of methane. With neither plant does the presence of nitrate offer any advantage over ammonium under anaerobic conditions with respect to the accumulation of potassium, or to the amount of growth as noted above.

Summary

The accumulation of bromide over a 24-hour absorption period by excised roots of rice at different oxygen pressures is similar to that of barley and tomato. The inflection in the accumulation curve of the three plants is reached at approximately 3 per cent. oxygen; at higher partial pressures of oxygen only small increases in bromide accumulation occur.

The net accumulation of potassium is of a similar character. Below 1 per cent. oxygen, however, there is a rapid and appreciable loss from the initial potassium content of barley and tomato, and rice releases potassium to a certain extent.

Under anaerobic conditions that prevent bromide accumulation by excised roots, rice roots with attached shoots accumulate appreciable amounts of bromide. The roots of entire barley plants show a smaller relative uptake of bromide, while intact tomato roots do not accumulate and respond like excised roots.

An atmosphere of pure carbon dioxide prevents accumulation by excised roots of all plants, and for whole plants it causes a similar inhibition accompanied by wilt of the shoot systems.

Plants grown aerobically through the seedling stage and transferred to soil culture show diverse response to drainage and submergence over a six-week period. With both loam and clay soils, submergence is lethal to the tomato, retards the growth of barley, but improves that of rice.

Plants grown aerobically through the seedling stage and transferred to water culture with continuous root exposure to air, carbon dioxide, nitrogen, and methane for six weeks follow the general relations indicated by soil cultures.

Compared to a control plant (unaerated), aeration of the root system improves the growth of tomato appreciably and of barley to a small degree. The growth of rice is not affected by aeration, but the root tips of this plant suffer a discoloration indicating injury.

The passage of carbon dioxide through the root medium results in an immediate wilting of all plants and no growth occurs.

Nitrogen gas reduces tomato shoot and root growth by about 90 per cent., while barley is decreased by 45 per cent. Rice is comparable to the control within the limits of experimental error.

Methane inhibits the growth of the tomato plant completely, while it reduces that of barley to a greater extent than does nitrogen. The root growth of rice in methane is superior to that of all other treatments, and 70 per cent. larger than the control.

Nitrate as a source of nutrient nitrogen and potentially of oxygen, does not increase the growth or potassium uptake of rice and barley compared to ammonium or ammonium nitrate, under anaerobic conditions of methane exposure to the roots.

THE UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. ARRINGTON, L. B., and SHIVE, J. W. Oxygen and carbon dioxide content of culture solutions in relation to cation and anion nitrogen absorption by tomato plants. Soil Sci. **42**: 341–357. 1936.
2. BERGMAN, H. F. The relation of aeration to the growth and activity of roots and its influence on the ecesis of plants in swamps. Ann. Bot. **34**: 13–33. 1920.
3. CANNON, W. A. Physiological features of roots with especial reference to the relations of roots to aeration of the soil. Carnegie Inst. Washington Publ. no. 368. 1925.
4. CLARK, H. E., and SHIVE, J. W. Influence of continuous aeration upon the growth of tomato plants in solution culture. Soil Sci. **34**: 37–41. 1925.
5. CONWAY, V. M. Aeration and plant growth in wet soils. Bot. Rev. **6**: 149–163. 1940.
6. DURELL, W. D. The effect of aeration on growth of the tomato in nutrient solution. Plant Physiol. **16**: 327–341. 1941.
7. FENN, W. O. A new method for the simultaneous determination of minute amounts of carbon dioxide and oxygen. Amer. Jour. Physiol. **84**: 110–118. 1928.
8. FREE, E. E. Effect of aeration on the growth of buckwheat in water culture. Johns Hopkins Univ. Cir. 293. 1917.
9. HAEHNEL, W., and MUGDAN, M. Estimation of oxygen with cuprammonium compounds. Zeitschr. angew. Chemie **33**: 35. 1920.
10. HALL, A. D., BRENCHLEY, W. E., and UNDERWOOD, L. M. The soil solution and the mineral constituents of the soil. Jour. Agr. Sci. **6**: 278–301. 1914.
11. HARRISON, W. H., and AIYER, P. A. S. The gases of swamp rice soils: Part I. Their composition and relationship to the crop. India Dept. Agr. Memoirs, Chem. Series **3**: 65–106. 1913–14.
12. ————, and ————. The gases of swamp rice soils: Part II. Their utilization for the aeration of the roots of the crop. India Dept. Agr. Memoirs, Chem. Series **4**: 1–17. 1914–16.
13. HOAGLAND, D. R., and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
14. LEATHER, J. W. Soil gases. India Dept. Agr. Memoirs, Chem. Series **4**: 85–134. 1914–1916.
15. RAALTE, M. H. VAN. On the oxygen supply of rice roots. Ann. Jard. Bot. Buitenzorg **50**: 99–113. 1940.
16. SETHI, R. L. Root development in rice under different conditions of growth. India Dept. Agr. Memoirs, Bot. Series **18**: 2–57. 1930.
17. SHIVE, J. W. The balance of ions and oxygen tension in nutrient substrates for plants. Soil Sci. **51**: 445–460. 1941.

18. STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. V. Observations upon the effects of time, oxygen, and salt concentration upon absorption and respiration by storage tissue. Protoplasma **18**: 208–242. 1933.

19. ————————, BERRY, W. E., and BROYER, T. C. The absorption and accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. **50**: 345–366. 1936.

20. STILES, W., and JØRGENSEN, I. Observations on the influence of aeration of the nutrient solution in water culture experiments, with some remarks on the water culture method. New Phytol. **16**: 181–197. 1917.

21. TREADWELL, F. P. Analytical chemistry. Vol. 2. John Wiley & Sons. New York. 1912.

22. WEAVER, J. E., and HIMMEL, W. J. Relation of increased water content and decreased aeration to root development in hydrophytes. Plant Physiol. **5**: 69–92. 1930.

EFFECTS OF IRON ON CHLOROPHYLLOUS PIGMENTS, ASCORBIC ACID, ACIDITY AND CARBOHYDRATES OF *ANANAS COMOSUS* (L.) MERR., SUPPLIED WITH NITRATE OR AMMONIUM SALTS[1]

C. P. SIDERIS AND H. Y. YOUNG

(WITH SIXTEEN FIGURES)

Introduction

In a former paper (**12**) data were presented on the effects of iron on growth, certain physiochemical properties, and ash constituents of *Ananas comosus* (L.) Merr. The present paper is concerned with data from the same plants pertaining to the amounts and distribution of chlorophyll, carotenoid pigments, titrable acidity, ascorbic acid, dextrose, levulose, sucrose, starch, the pentosan and hexosan fractions of hemicellulose, cellulose and lignins in different sections of the leaves, stem, and roots.

Methods

Sixteen plants were grown for 12 months in constantly aerated solution cultures supplied with equal amounts of nitrate- or ammonium-nitrogen and either with or without iron (5 mg. per liter of solution). At the end of this period 8 were harvested, weighed, sectioned, and analyzed following the technique reported in a former paper (**10**). Methods employed for the chemical analyses of the substances mentioned in this paper were as follows.

The chlorophyllous pigments were determined according to SCHERTZ'S methods (**8, 9**). The titrable acidity of the tissues, expressed as percentage of citric acid, was determined with a 0.1 N solution of sodium hydroxide on a 10-ml. aliquot representing 2 grams of fresh tissue. Ascorbic acid was determined with 2, 6-dichlorophenolindophenol in acetic acid according to the method of BESSEY and KING (**3**). Sugars in the aqueous extracts of fresh tissues were determined by the method of QUISUMBING and THOMAS (**7**) after treatment with lead acetate and deleading with disodium phosphate. Dextrose was determined by the hypoiodite method of WIDDOWSON (**14**), and levulose was calculated from the difference between the values of reducing sugars and dextrose. The colorimetric method of PUCHER and VICKERY (**6**) was employed for the determination of starch. Hemicelluloses were determined after hydrolysis with 0.75 N hydrochloric acid for 2.5 hours on the residue of the sample employed for the analysis of starch following the removal of all dissolved substances. The sugars in the above hydrolysate, reported as total hemicelluloses, contained mixtures of pentose and hexose sugars. The furfural content of the total hemicellulose sugars was made the basis for the separation of the pentose from the hexose sugars. The residue

[1] Published with the approval of the Acting Director as Technical Paper no. 149 of the Pineapple Research Institute, University of Hawaii.

of the sample of tissues from the hemicellulose determination was employed for determining lignin and cellulose. Lignin was determined by the method of PETERSON, WALDE and HIXON (**5**). Cellulose was calculated as the difference between the weights of the dried residue (after the extraction of hemicelluloses) and lignin.

The significance of the differences between the amounts of the various items of the different cultures was calculated, as reported in a former publication (**12**), from CONRAD'S modification of BESSEL'S formula, and the values of z were obtained from Student's Table as modified by LOVE. Table VII reports the statistical significance of the difference of the amounts of the various items mentioned.

Results

Synoptic expressions have been introduced in the presentation of the results and in the discussion to replace the longer ones for the appellation of

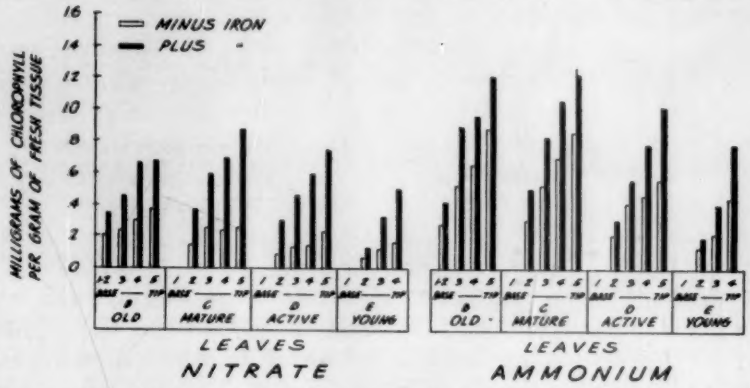


FIG. 1. Chlorophyll in pineapple tissues of plants grown with and without iron, and with nitrate or ammonium as source of nitrogen.

the various treatments. The series Ammonium-nitrogen will be designated by A-n and Nitrate-nitrogen by N-n. Also, the cultures "Plus-iron" by F and Minus-iron" by 0.

CHLOROPHYLLOUS PIGMENTS

Chlorophyll was separated from the carotenoid pigments but no attempt was made to separate either chlorophyll alpha from chlorophyll beta or carotene from xanthophyll. The data in table I and in figure 1, show that the plants of the F cultures contained amounts of chlorophyll considerably in excess of those of the 0 cultures regardless of the source of nutrient nitrogen. They show, also, that the plants of the A-n series contained greater quantities of chlorophyll than those of the N-n series. This relationship has already been discussed (**10**).

Carotenoids were higher in the plants of the F than 0 cultures of both series. They ranged from 10 to 30 per cent. in the A-n and from 70 to 180 per cent. in the N-n series. Such comparisons show that the distribution of carotenoids paralleled that of chlorophylls. Since the amounts of both types

TABLE I

MILLIGRAMS OF CHLOROPHYLL, CAROTENOIDS, CITRIC ACID (TITRABLE ACIDITY), AND ASCORBIC ACID PER GRAM OF FRESH TISSUE AND pH VALUES OF THE EXTRACTED SAP IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES OF NITROGEN

PLANT SECTIONS	MINUS IRON					PLUS IRON				
	CHLOROPHYLL	CAROTENOIDS	CITRIC ACID	ASCORBIC ACID	pH	CHLOROPHYLL	CAROTENOIDS	CITRIC ACID	ASCORBIC ACID	pH
	mg.	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	*mg.*	
Leaves										
Old (B) 1+2 (base)	0.20	0.020	2.16	0.29	5.2	0.33	0.033	1.08	0.02	5.3
3	0.23	0.034	7.54	3.03	4.1	0.46	0.054	9.34	0.91	3.9
4	0.30	0.042	10.43	3.90	3.9	0.66	0.079	12.92	1.49	3.7
5 (tip)	0.37	0.051	11.12	4.73	3.9	0.67	0.079	14.00	1.49	3.6
Mature (C) 1 (base)	.....	.....	0.72	0.01	5.5	.....	.....	0.38	0.01	5.6
2	0.12	0.012	3.60	0.53	4.4	0.35	0.031	2.88	0.05	4.3
3	0.25	0.031	9.00	2.31	4.0	0.59	0.067	8.58	0.57	3.9
4	0.23	0.036	11.50	3.48	3.8	0.69	0.083	10.80	1.85	3.7
5 (tip)	0.25	0.038	11.50	5.28	3.9	0.86	0.106	13.64	2.09	3.5
Active (D) 1 (base)	.....	.....	0.71	0.01	5.5	.....	.....	0.38	0.01	5.6
2	0.08	0.006	2.88	0.14	4.4	0.28	0.022	1.79	0.01	4.5
3	0.13	0.011	10.42	0.88	3.8	0.46	0.047	11.15	0.10	3.6
4	0.18	0.025	14.50	3.35	3.6	0.60	0.073	16.50	2.12	3.4
5 (tip)	0.23	0.037	16.20	5.61	3.4	0.74	0.104	20.15	2.87	3.4
Young (E) 1 (base)	.....	.....	1.09	0.01	5.5	.....	.....	0.70	0.01	5.7
2	0.07	0.004	2.52	0.04	4.6	0.12	0.009	2.52	0.01	4.4
3	0.13	0.013	11.90	0.77	3.4	0.33	0.032	13.70	0.16	3.4
4+5 (tip)	0.17	0.026	16.56	2.97	3.3	0.50	0.067	22.65	1.10	3.3
Stem										
Base	0.0	0.0	1.44	0.0	5.3	0.0	0.0	1.44	0.0	5.4
Middle	0.0	0.0	2.16	0.0	5.4	0.0	0.0	2.52	0.0	5.3
Apex	0.0	0.0	2.52	0.0	5.3	0.0	0.0	2.16	0.0	5.5
Roots	0.0	0.0	0.36	0.0	5.4	0.0	0.0	0.32	0.0	5.6

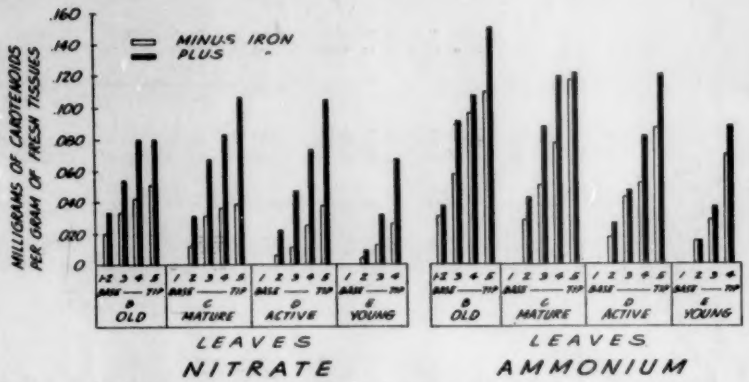


FIG. 2. Carotenoid content of pineapple tissues of plants grown with and without iron, and with nitrate or ammonium nitrogen.

of pigments present in a given section were not proportional to the amounts of iron supplied in the nutrient solution, it is probable that the degree of availability rather than the total amounts of iron within the plants were the determinant factors for the concentrations of chlorophyll and carotenoids. Iron availability was greater in the A-n than N-n series because of more favorable H-ion concentrations in the solution cultures of the former series as shown in an earlier publication (**12**).

ACIDITY

Titrable acidity is reported as milligrams of citric acid per gram of fresh tissue in tables I and II, and in figure 3. The values differed in different sections and also varied to some extent in comparable sections of the plants of the different cultures. They were generally greater in the chlorophyllous

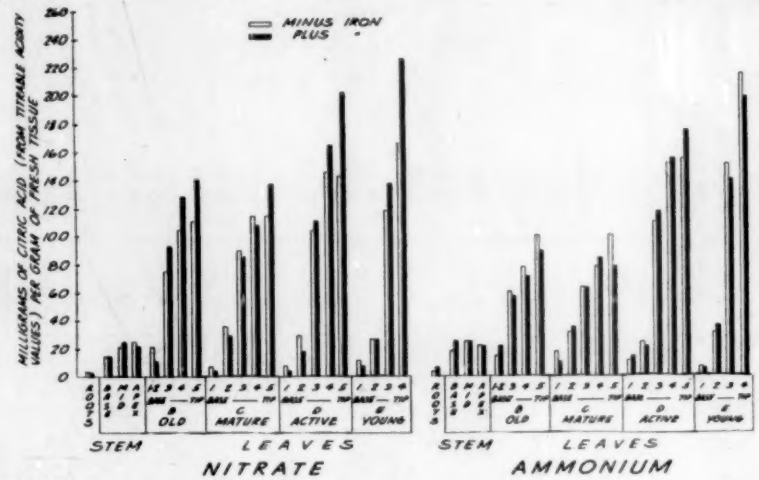


FIG. 3. Citric acid content of pineapple tissues of plants grown with and without iron, and with nitrate or ammonium nitrogen.

TABLE II

MILLIGRAMS OF CHLOROPHYLL, CAROTENOIDS, CITRIC ACID (TITRABLE ACIDITY), AND ASCORBIC ACID PER GRAM OF FRESH TISSUE AND pH VALUES OF THE EXTRACTED SAP IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES OF NITROGEN

Plant sections	Minus iron					Plus iron				
	Chlorophyll	Carotenoids	Citric acid	Ascorbic acid	pH	Chlorophyll	Carotenoids	Citric acid	Ascorbic acid	pH
	mg.	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	*mg.*	
Leaves										
Old (B) 1+2 (base)	0.27	0.031	1.44	0.01	5.3	0.42	0.037	2.16	0.02	5.0
3	0.53	0.057	6.12	1.77	4.1	0.90	0.091	5.77	1.23	4.2
4	0.66	0.096	7.90	2.75	3.9	0.96	0.107	7.17	1.84	3.9
5 (tip)	0.89	0.109	10.05	3.36	3.7	1.21	0.150	8.96	2.27	3.8
Mature (C) 1 (base)	.....	.....	1.79	0.01	5.4	.....	.....	1.02	0.01	5.2
2	0.30	0.028	3.23	0.28	4.6	0.50	0.042	3.59	0.04	4.6
3	0.53	0.050	6.46	1.22	4.0	0.83	0.087	6.40	1.35	4.2
4	0.70	0.077	7.92	2.93	3.8	1.05	0.118	8.57	2.65	3.9
5 (tip)	0.86	0.116	10.10	4.34	3.6	1.22	0.120	7.90	2.88	3.8
Active (D) 1 (base)	.....	.....	1.09	0.01	5.4	.....	.....	1.44	0.01	5.4
2	0.21	0.017	2.51	0.01	4.3	0.30	0.026	2.16	0.01	4.6
3	0.41	0.042	11.13	0.51	3.6	0.56	0.046	11.85	0.86	3.8
4	0.47	0.051	15.10	2.93	3.5	0.78	0.081	15.50	3.39	3.7
5 (tip)	0.56	0.066	15.50	5.04	3.4	1.02	0.119	17.60	6.23	3.7
Young (E) 1 (base)	.....	.....	0.72	0.01	5.6	.....	.....	0.64	0.01	5.6
2	0.13	0.015	3.23	0.01	4.3	0.20	0.014	3.74	0.01	4.7
3	0.22	0.028	15.10	0.25	3.4	0.40	0.036	14.00	0.30	3.4
4+5 (tip)	0.44	0.069	21.60	2.75	3.3	0.78	0.087	19.90	2.47	3.4
Stem										
Base	0.0	0.0	1.79	0.0	5.2	0.0	0.0	2.52	0.0	5.4
Middle	0.0	0.0	2.52	0.0	5.2	0.0	0.0	2.52	0.0	5.2
Apex	0.0	0.0	2.22	0.0	5.3	0.0	0.0	2.18	0.0	5.2
Roots	0.0	0.0	0.32	0.0	6.0	0.0	0.0	0.64	0.0	5.7

TABLE III

MILLIGRAMS OF TOTAL AND REDUCING SUGARS AND THEIR FRACTIONS OF DEXTROSE, LEVULOSE, SUCROSE, AND STARCH PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- AND MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES OF NTROGEN

Plant sections	Minus iron						Plus iron					
	Total sugars	Reducing sugars	Dextrose	Levulose	Sucrose	Starch	Total sugars	Reducing sugars	Dextrose	Levulose	Sucrose	Starch
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves												
Old (B) 1+2 (base)	20.7	18.8	12.6	6.2	1.9	1.3	19.6	15.7	11.0	4.7	3.9	1.9
3	19.6	18.5	9.9	8.6	1.1	1.2	18.1	14.3	9.0	5.3	3.8	2.1
4	19.3	18.2	10.8	7.4	1.1	1.1	19.3	15.6	10.4	5.2	3.7	1.4
5 (tip)	19.3	18.0	11.7	6.3	1.3	1.2	21.6	17.6	12.8	4.8	4.0	1.7
Mature (C) 1 (base)	24.0	22.5	15.8	6.7	1.5	1.5	25.9	21.4	13.3	8.1	4.5	2.2
2	21.0	19.8	12.2	7.6	1.2	3.5	22.1	17.3	12.4	4.9	4.8	6.3
3	12.5	9.6	6.1	3.5	2.9	2.5	16.2	11.3	7.0	4.3	4.9	2.9
4	14.1	10.6	7.5	3.1	3.5	2.3	16.8	11.7	8.4	3.3	5.1	1.7
5 (tip)	18.7	14.7	9.9	4.8	4.0	2.9	21.5	13.7	10.8	2.9	7.8	2.9
Active (D) 1 (base)	24.7	22.7	15.8	6.9	2.0	3.4	25.5	22.4	16.2	6.2	3.1	3.5
2	21.3	19.5	11.9	7.6	1.8	4.2	22.9	18.1	12.6	5.5	4.8	4.7
3	17.4	14.0	7.2	6.8	3.4	3.0	21.9	15.2	8.1	7.1	6.7	3.5
4	17.0	12.5	6.8	5.7	4.5	1.8	22.1	15.7	10.4	5.3	6.4	1.5
5 (tip)	17.7	12.2	8.1	4.1	5.5	1.8	24.6	16.5	11.7	4.8	8.1	1.8
Young (E) 1 (base)	14.1	13.1	8.1	5.0	1.0	0.7	15.5	12.5	9.5	3.0	3.0	0.8
2	14.3	14.3	8.3	6.0	0.0	0.2	15.3	14.1	9.0	5.1	1.2	0.2
3	19.8	18.6	8.1	10.5	1.2	1.5	21.4	17.5	8.1	9.4	3.9	1.4
4+5 (tip)	23.3	19.4	9.0	10.4	1.9	1.1	26.9	18.6	9.2	9.4	8.3	1.4
Stem												
Base	16.6	12.6	14.2	0.0	4.0	17.7	17.0	13.6	14.4	0.0	3.4	19.9
Middle	12.2	7.6	8.4	0.0	4.6	38.6	13.1	8.3	8.7	0.0	4.8	43.7
Apex	17.7	10.4	9.6	0.8	7.3	25.8	19.6	12.1	11.0	1.1	7.5	45.9
Roots	4.4	1.2	1.8	0.0	3.2	0.0	4.3	1.8	1.6	0.2	2.5	0.0

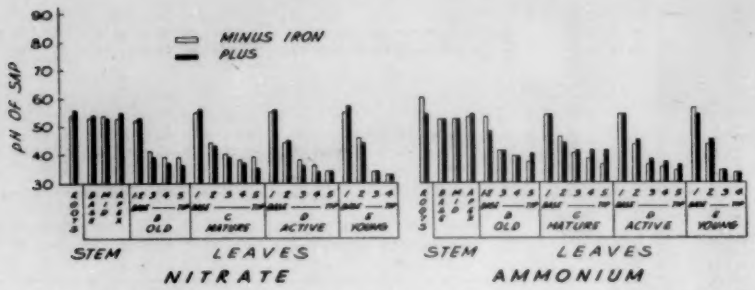


FIG. 4. Active acidity (pH) of sap of pineapple tissues of plants grown with and without iron, and with nitrate or ammonium nitrogen.

than non-chlorophyllous sections of the leaves, a fact also reported in a previous publication (**10**). The values of titrable acidity fluctuated considerably between comparable sections in the plants of the different cultures. They were not consistent with respect to either the amounts of iron or the source of nitrogen. In the N-n series, however, the cholorphyllous sections of the leaves of the F cultures contained significantly greater amounts of titrable acidity than those of the 0 cultures, yet in the A-n the differences in acidity between the F and 0 plants were not statistically significant.

The pH values of the sap, reported in tables I and II and figure 4, were in relative agreement with those of the acidity for corresponding sections although the great amounts of buffer substances in certain sections made perfect agreement between titrable acidity and pH values impossible.

ASCORBIC ACID

The ascorbic acid content of the sap, reported in tables I and II, and in figure 5, was higher in the chlorophyllous than in the non-chlorophyllous sections, thus approximately paralleling the distribution of citric acid. The stem and roots either completely lacked ascorbic acid or the amounts were very small and insignificant.

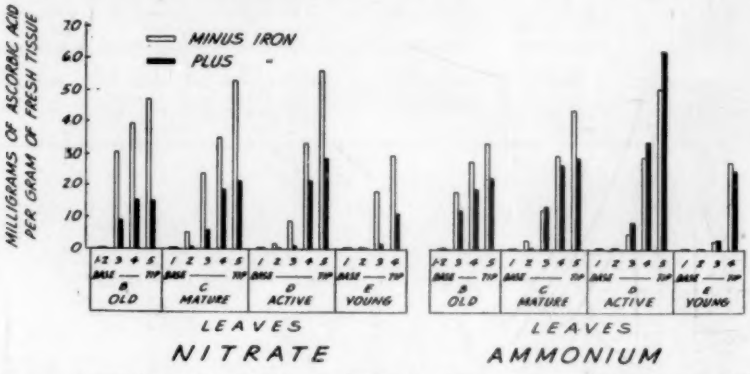


FIG. 5. Ascorbic acid content of pineapple tissues from plants grown with and without iron, with nitrate or ammonium nitrogen.

Ascorbic acid values were considerably greater in the leaves of the 0 than F cultures of the N-n series, the differences being highly significant, as reported in table VII. In the plants of the A-n series ascorbic acid was apparently higher in the "old" (B) and "mature" (C) groups of leaves of the 0 than F cultures, but not in the "active" (D) and "young" (E) groups. Ascorbic acid showed in many sections of the N-n series an inverse relationship to citric acid whereas in the A-n series the relationship was direct.

Total sugars

The amounts of total sugars, reported in tables III and IV, and in figure 6, were, with few exceptions, higher for the plants of the F than 0 cultures

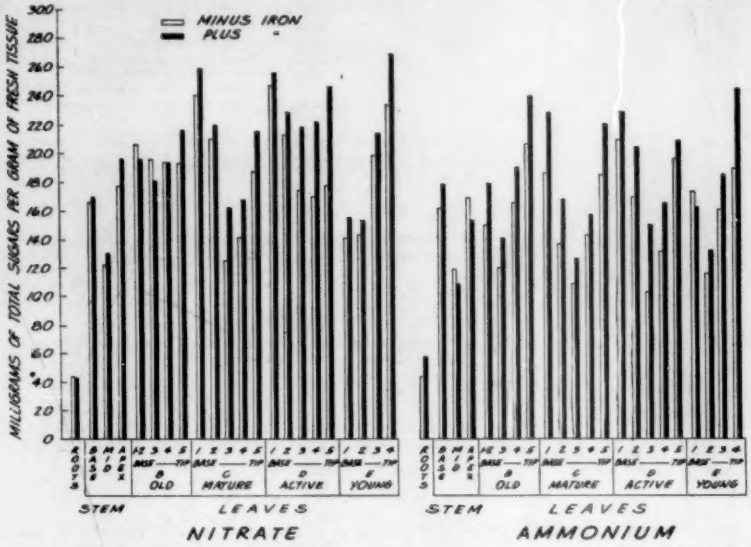


FIG. 6. Total sugars in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

of both series. They were higher in the basal and apical sections of the stem than in the medial sections. The amounts were greater in the N-n than A-n series. Contrasts between the amounts of sugars and chlorophyll in the "mature" (C) and "active" (D) leaves of the F and 0 cultures of the N-n series show that mean chlorophyll values were 212 per cent. (F = 0.572 and 0 = 0.183) and sugars 16.6 per cent. (F = 27.5 and 0 = 23.5) higher in the F than 0 cultures. Variability in the chlorophyll values was 30.8 per cent. $\left(\text{CV} = \frac{0.12 \times 100}{0.39}\right)$, whereas in the sugars it was 57.4 $\left(\text{CV} = \frac{2.236 \times 100}{3.9}\right)$. Accordingly, sugar values were 1.86 times ($57.4 \div 30.8 = 1.86$) more variable, on the basis of these calculations, than chlorophyll values. The results suggest, therefore, that sugar accumulations in the leaves had been influenced by other factors intimately associated with the metabolic and photosynthetic activities of the plant besides chlorophyll concentrations.

TABLE IV

MILLIGRAMS OF TOTAL AND REDUCING SUGARS AND THEIR FRACTIONS OF DEXTROSE, LEVULOSE, SUCROSE, AND STARCH PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- AND MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES OF NITROGEN

Plant sections	Minus iron						Plus iron					
	Total sugars	Reducing sugars	Dextrose	Levulose	Sucrose	Starch	Total sugars	Reducing sugars	Dextrose	Levulose	Sucrose	Starch
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves												
Old (B) 1+2 (base)	15.0	12.0	9.6	2.4	3.0	0.1	17.8	16.5	10.1	6.4	1.3	0.4
3	12.0	8.3	6.6	1.7	3.7	0.3	14.1	11.8	7.8	4.0	2.3	0.6
4	16.6	11.7	10.0	1.7	4.9	0.3	19.1	14.3	10.4	3.9	4.8	0.8
5 (tip)	20.7	14.6	12.4	2.2	6.1	0.3	24.0	18.2	13.3	4.9	5.8	1.0
Mature (C) 1 (base)	18.6	16.6	12.2	4.4	2.0	0.1	22.9	21.7	13.7	8.0	1.2	0.3
2	13.7	11.1	8.2	2.9	2.6	0.3	16.8	15.0	9.5	5.5	1.8	1.0
3	10.9	7.6	7.4	0.2	3.3	0.6	12.7	9.1	6.1	3.0	3.6	1.3
4	14.3	9.9	8.7	1.2	4.4	1.0	15.7	11.0	7.9	3.1	4.7	1.9
5 (tip)	18.5	12.6	11.3	1.3	5.9	1.5	22.1	15.1	11.7	3.4	7.0	3.0
Active (D) 1 (base)	20.9	18.9	12.6	6.3	2.0	0.2	22.9	22.3	13.3	9.0	0.6	0.7
2	16.9	15.3	9.7	5.6	1.6	0.2	20.4	18.9	10.6	8.3	1.5	1.1
3	10.3	6.9	4.6	2.3	3.4	0.5	15.0	11.0	5.5	5.5	4.0	1.2
4	13.2	7.0	5.1	0.9	6.2	0.8	16.8	10.2	7.8	2.4	6.6	1.1
5 (tip)	19.6	11.1	9.6	1.5	8.5	1.4	20.9	12.1	10.2	1.9	8.8	1.9
Young (E) 1 (base)	17.3	16.1	10.4	5.7	1.2	0.2	16.3	14.5	9.0	5.5	1.8	0.3
2	11.6	10.7	5.8	4.9	0.9	0.1	13.2	12.7	6.9	5.8	0.5	0.0
3	16.1	11.9	5.7	6.2	4.2	0.3	18.5	15.3	7.0	8.5	3.2	0.3
4+5 (tip)	18.9	11.1	6.6	4.5	7.8	0.7	24.5	17.1	9.7	7.4	7.4	0.5
Stem												
Base	16.2	13.6	13.5	0.1	2.6	2.5	17.8	14.4	13.7	0.7	3.4	5.9
Middle	11.9	7.8	7.7	0.1	4.1	7.8	10.8	7.5	6.9	0.6	3.3	17.7
Apex	17.0	10.8	8.7	2.1	6.2	10.5	15.3	9.8	8.5	1.3	5.5	18.3
Roots	4.3	2.5	2.5	0.0	2.0	0.0	5.7	3.3	2.5	0.8	2.4	0.0

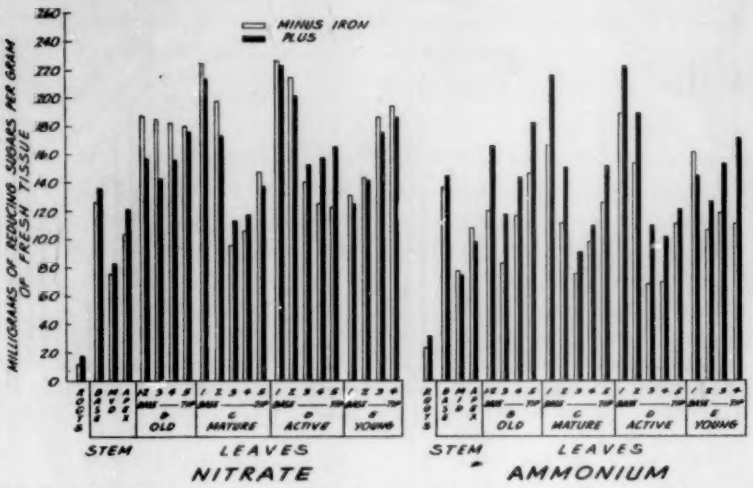


FIG. 7. Reducing sugars in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

REDUCING SUGARS

Data for reducing sugars are presented in figure 7 and for dextrose and levulose in figures 8 and 9, and in tables III and IV. Dextrose occurred in much greater amounts in practically all the sections of the leaves, stem, and roots than did levulose. In the stem, levulose was either absent or present in very small amounts while dextrose made up the bulk of the amounts of

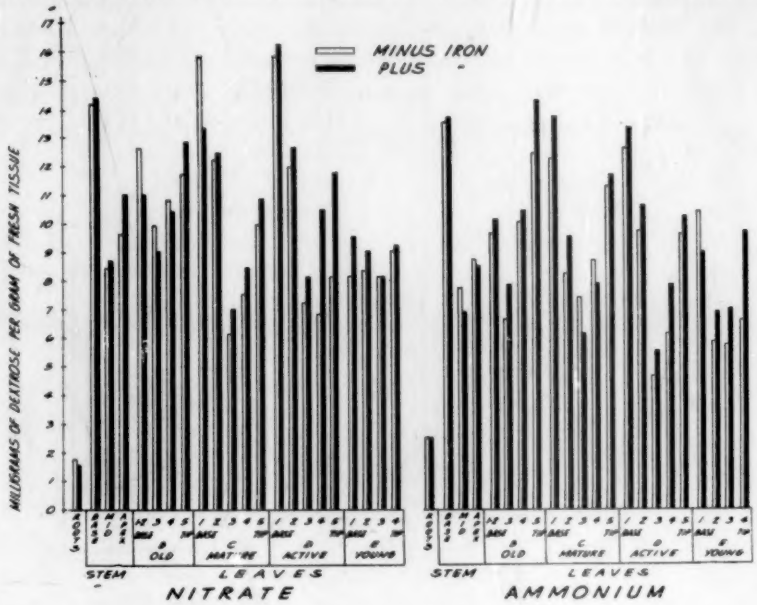


FIG. 8. Dextrose in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

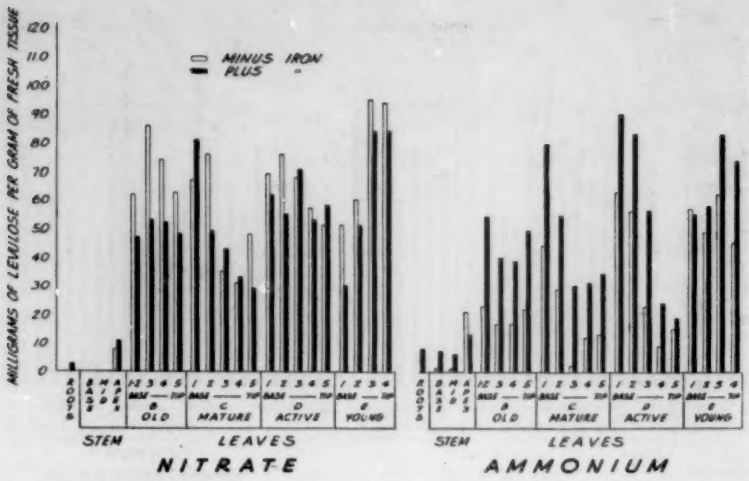


FIG. 9. Levulose in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

reducing sugars. Dextrose accumulated mostly in the basal and terminal sections of the leaves of the "old" (B), "mature" (C), and "active" (D) groups, whereas levulose accumulations were highest in the basal sections. In the leaves of the "young" (E) group the differences between the amounts of either dextrose or levulose of the basal and terminal sections were not as great as in the other groups of leaves. Dextrose occurred in greater amounts in the chlorophyllous (2, 3, 4, 5), while levulose was more abundant in the basal (1), non-chlorophyllous sections of the leaves. The low amounts of levulose in the chlorophyllous and the high in the non-chlorophyllous sections might indicate that either the rate of levulose synthesis was consider-

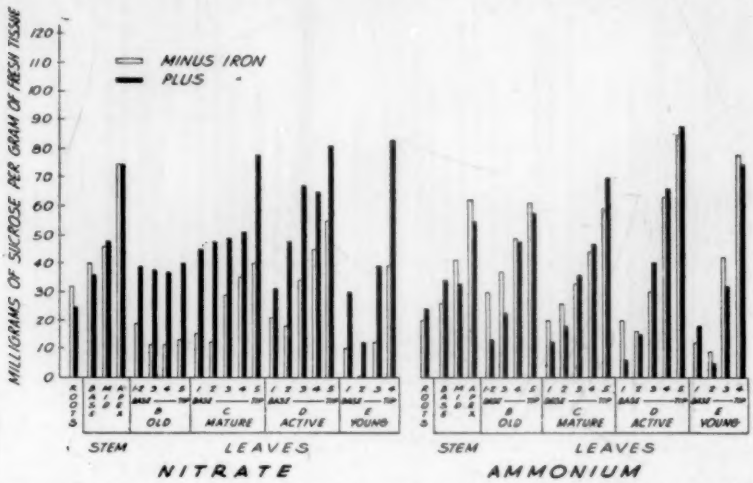


FIG. 10. Sucrose in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

ably lower than that of dextrose or that it was utilized more in the syntheses of various types of substances.

Sucrose

The data in tables III and IV, and in figure 10 show that sucrose was more abundant in the plants of the F than 0 cultures of the N-n series. In the A-n series sucrose did not accumulate in the F cultures and in many sections the 0 cultures had slightly higher sucrose values. There can be no doubt, however, that the greater amounts of sucrose in the plants of the F than 0 cultures of the N-n series resulted from different rates of carbohydrate synthesis due to appreciable differences in chlorophyll content. The lack of

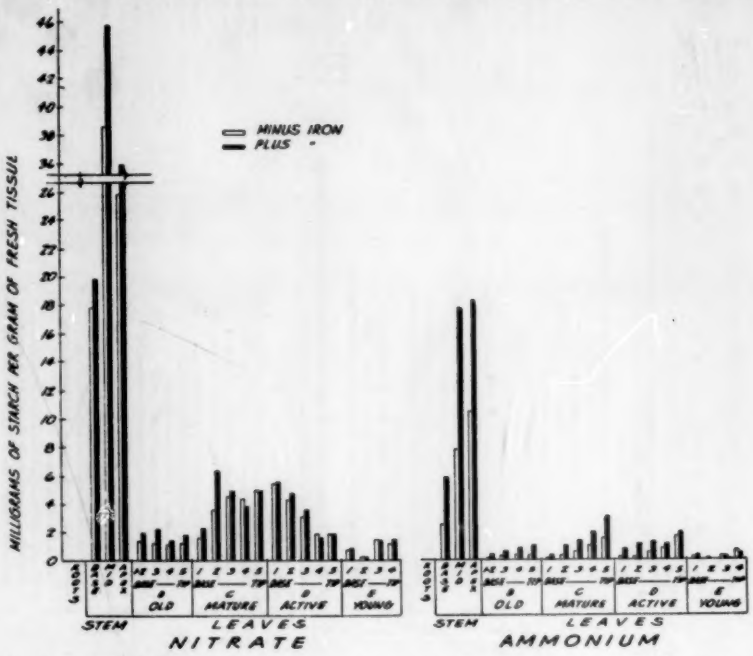


Fig. 11. Starch in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

appreciable difference in the sucrose content of the F and 0 cultures in the A-n series may be attributed to the small differences in the chlorophyll content and possibly to different rates of utilization of sucrose.

Starch

Iron availability and the source of nitrogen had affected the amounts of starch more than any other carbohydrates, as reported in tables III and IV, and in figure 11. The plants of the F cultures of both series contained, with few minor exceptions, more starch than those of the 0 cultures. Also, starch accumulations were enhanced more by nitrate- than ammonium-nitrogen under the same concentrations of iron.

Starch accumulations were restricted mostly to the tissues of the stem and were appreciably greater in the N-n than in the A-n series, whereas reducing sugars and sucrose accumulated mostly in the leaves although some stem sections contained as high amounts of sugars as did leaf sections. The "mature" (C) and "active" (D) leaves contained greater amounts of starch than did those of the "old" (B) and "young" (E) groups. The small amounts of starch in the leaves of the "young" (E) group can be explained on the basis of a relatively rapid utilization of carbohydrates for the synthesis of new tissues, while those in the leaves of the "old" (B) group may be explained by a decreased rate of photosynthetic activity or by a possible hydrolysis of starch resulting from tissue senility.

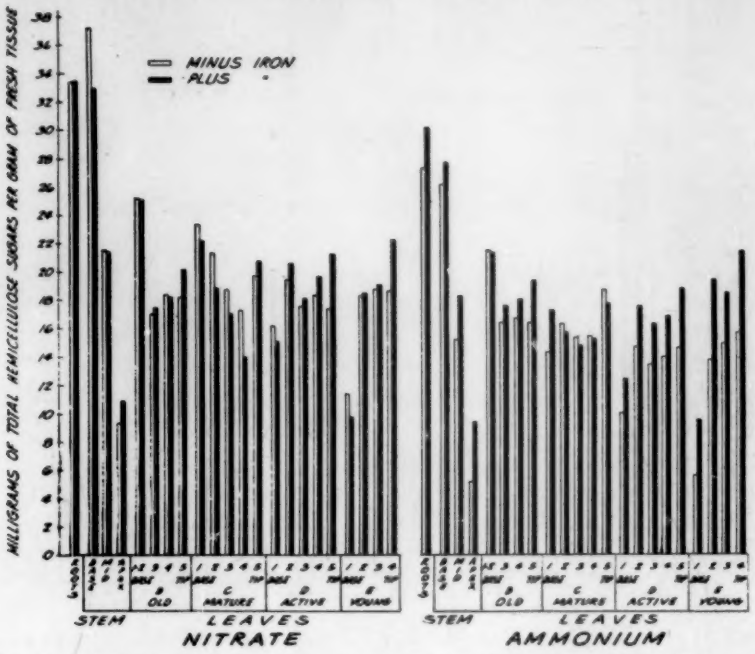


FIG. 12. Total hemicellulose sugars in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

A synoptic analysis of the effects of iron and nitrogen sources on starch shows that nitrate-nitrogen versus ammonium-nitrogen increased the average starch values in the stem 161.6 per cent. for the plus-iron and 295.0 per cent. for the minus-iron cultures. The gains of starch in the stem of the plus-iron versus minus-iron cultures were 33.2 per cent. for the nitrate series and 101.3 per cent. for the ammonium series.

HEMICELLULOSES

The amounts of total hemicellulose sugars, reported in tables V and VI, and in figures 12, 13 and 14, were slightly greater in the F than 0 cultures.

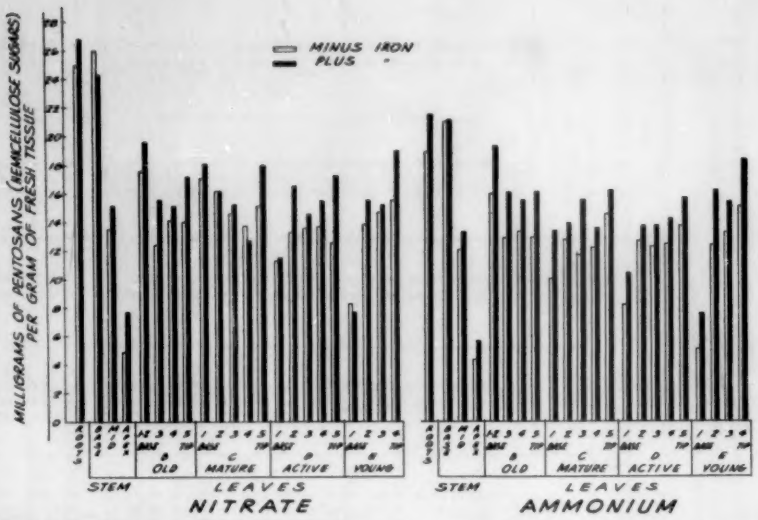


FIG. 13. Pentosans in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

Pentose sugars, constituting more than three-fourths the amounts of total hemicellulose sugars, were generally higher in most leaf sections of the F than 0 cultures of both series. The amounts of hexose sugars showed decided deviations from pentose sugars, being generally higher in the leaves of the 0 than F cultures of the N-n series. In the A-n series the amounts of hexose sugars were higher in the "old" (B) and "mature" (C) and lower in the "active" (D) and "young" (E) groups of leaves of the 0 than in the F cultures. The amounts of hexose sugars in the stem were higher in the 0 than in the F cultures of the N-n series, while in the A-n series the values

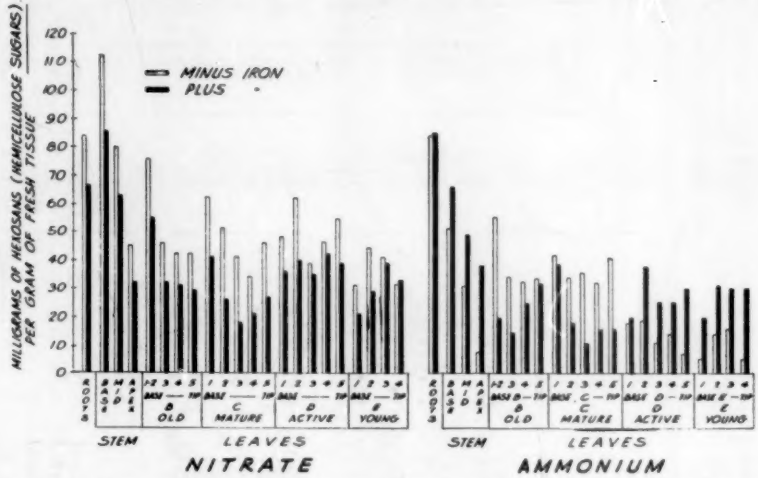


FIG. 14. Hexosans in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

TABLE V

MILLIGRAMS OF TOTAL HEMICELLULOSES AND THEIR FRACTIONS OF PENTOSANS AND HEXOSANS, CELLULOSES, AND LIGNIN PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- AND MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES OF NITROGEN

Plant sections	Minus iron					Plus iron				
	Hemicelluloses			Celluloses	Lignin	Hemicelluloses			Celluloses	Lignin
	Total	Pentosans	Hexosans			Total	Pentosans	Hexosans		
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves										
Old (B) 1+2 (base)	25.2	17.6	7.6	39.1	8.9	25.1	19.6	5.5	39.6	9.6
3	17.0	12.4	4.6	27.8	7.7	17.4	15.6	1.8	28.8	8.4
4	18.3	14.1	4.2	28.2	9.1	18.2	15.1	3.1	27.4	8.0
5 (tip)	18.2	14.0	4.2	29.0	10.3	20.1	17.2	2.9	30.8	9.9
Mature (C) 1 (base)	23.3	17.1	6.2	34.8	5.5	22.2	18.1	4.1	33.2	6.0
2	21.3	16.2	5.1	37.0	6.9	18.8	16.2	2.6	34.3	6.9
3	18.7	14.6	4.1	30.0	7.5	17.0	15.2	1.8	29.8	7.6
4	17.2	13.8	3.4	28.5	7.7	14.8	12.7	2.1	25.6	7.5
5 (tip)	19.7	15.1	4.6	30.9	8.9	20.7	18.0	2.7	33.8	10.6
Active (D) 1 (base)	16.1	11.3	4.8	30.2	4.6	15.1	11.5	3.6	25.4	4.9
2	19.4	13.3	6.2	37.1	6.5	20.6	16.6	4.0	39.7	8.2
3	17.5	13.6	3.9	31.3	7.5	18.1	14.6	3.5	34.2	8.4
4	16.3	13.7	4.6	28.9	9.6	19.7	15.5	4.2	34.2	9.9
5 (tip)	17.4	12.6	5.4	31.5	9.8	21.2	17.3	3.9	34.2	11.1
Young (E) 1 (base)	11.4	8.3	3.1	19.8	2.8	9.8	7.7	2.1	16.5	2.3
2	18.3	13.9	4.4	32.0	7.0	18.5	15.6	2.9	30.1	6.9
3	18.8	14.7	4.1	29.8	8.5	19.1	15.2	3.9	33.4	8.8
4+5 (tip)	18.7	15.6	3.1	31.8	10.0	22.3	19.1	3.2	37.1	10.7
Stem										
Base	37.2	26.0	11.2	37.2	18.0	32.9	24.3	8.6	34.4	13.1
Middle	21.5	13.5	8.0	21.0	5.0	21.4	15.1	6.3	21.4	4.9
Apex	9.3	4.8	4.5	11.4	1.0	10.9	7.7	3.2	13.5	1.3
Roots	33.4	25.0	8.4	43.5	28.8	33.5	26.8	6.7	50.1	30.9

TABLE VI

MILLIGRAMS OF TOTAL HEMICELLULOSES AND THEIR FRACTIONS OF PENTOSANS AND HEXOSANS, CELLULOSES, AND LIGNIN PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- AND MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES OF NITROGEN

Plant sections	Minus iron					Plus iron				
	Hemicelluloses			Celluloses	Lignin	Hemicelluloses			Celluloses	Lignin
	Total	Pentosans	Hexosans			Total	Pentosans	Hexosans		
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves										
Old (B) 1+2 (base)	21.6	16.1	5.5	29.6	14.6	21.5	19.5	2.0	33.6	12.1
3	16.4	13.0	3.4	31.6	9.2	17.7	16.2	1.5	30.2	9.4
4	16.7	13.4	3.3	28.8	10.1	18.1	15.6	2.5	28.7	11.3
5 (tip)	16.4	13.0	3.4	30.4	11.3	19.4	16.2	3.2	31.2	10.9
Mature (C) 1 (base)	14.3	10.1	4.2	25.8	4.5	17.4	13.5	3.9	27.2	5.0
2	16.3	12.9	3.4	30.2	7.1	15.8	14.0	1.8	35.4	7.3
3	15.4	11.8	3.6	27.6	8.3	14.8	13.7	1.1	28.2	8.3
4	15.5	12.3	3.2	28.4	8.8	15.3	13.7	1.6	28.4	7.9
5 (tip)	18.8	14.7	4.1	32.0	10.1	17.9	16.3	1.6	31.0	9.8
Active (D) 1 (base)	10.1	8.3	1.8	23.6	4.2	12.5	10.5	2.0	24.8	3.4
2	14.7	12.8	1.9	33.7	7.0	17.7	13.9	3.8	33.2	6.8
3	13.5	12.4	1.1	30.4	8.4	16.4	13.9	2.5	28.6	7.5
4	14.0	12.6	1.4	27.1	8.1	16.9	14.4	2.5	27.8	8.9
5 (tip)	14.6	13.9	0.7	32.5	9.9	18.9	15.9	3.0	29.2	9.4
Young (E) 1 (base)	5.7	5.2	0.5	16.1	2.4	9.7	7.7	2.0	14.2	2.0
2	13.9	12.5	1.4	29.5	7.1	19.5	16.4	3.1	29.1	7.0
3	15.0	13.4	1.6	30.7	9.5	18.6	15.6	3.0	29.6	8.5
4+5 (tip)	15.8	15.3	0.5	31.9	10.7	21.8	18.6	3.0	33.2	9.7
Stem										
Base	26.2	21.1	5.1	27.1	18.2	27.8	21.2	6.6	29.2	10.4
Middle	15.2	12.1	3.1	13.9	9.7	18.3	13.4	4.9	19.5	4.2
Apex	5.2	4.4	0.8	10.4	2.1	9.5	5.7	3.8	11.2	1.1
Roots	27.4	19.0	8.4	31.6	19.9	30.1	21.6	8.5	32.5	18.8

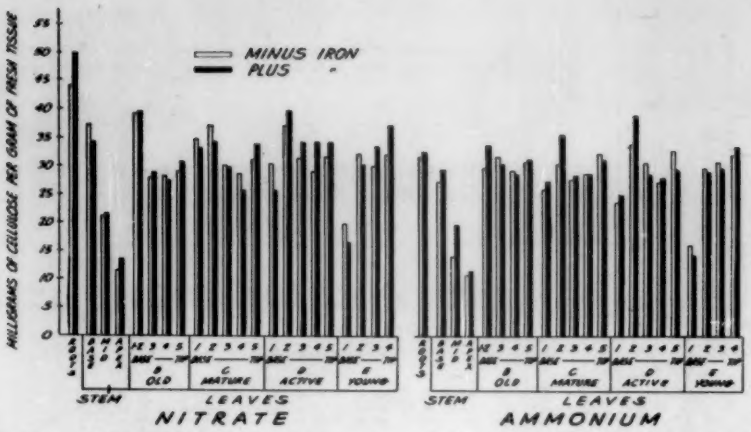


FIG. 15. Cellulose in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

were reversed. The results indicate that hexosans were more susceptible to variations by the difference in the amounts of iron and sources of inorganic nitrogen than pentosans.

CELLULOSE

The amounts of cellulose, reported in tables V and VI, and in figure 15, were affected very little by iron concentrations. The effects of the source of nitrogen in the solution culture on the amounts of cellulose in the plants of

TABLE VII

CALCULATED STATISTICAL SIGNIFICANCE* OF THE DIFFERENCE OF THE MEANS OF THE SUBSTANCES LISTED BELOW OF COMPARABLE SECTIONS OF THE LEAVES AND STEM OF PLANTS GROWN EITHER IN PLUS-IRON OR IN MINUS-IRON CULTURES AND SUPPLIED WITH NITRATE OR AMMONIUM SALTS AS SOURCES OF NITROGEN

SUBSTANCES	NITRATE-N				AMMONIUM-N			
	LEAVES		STEM		LEAVES		STEM	
	SIGNIFICANCE	IN FAVOR OF CULTURE	SIGNIFICANCE	IN FAVOR OF CULTURE	SIGNIFICANCE	IN FAVOR OF CULTURE	SIGNIFICANCE	IN FAVOR OF CULTURE
Chlorophyll	9999 : 1	+ Fe	.....	.....	9999 : 1	+ Fe	.....	.....
Carotenoids	9999 : 1	+ Fe	.....	.....	9999 : 1	+ Fe	.....	.....
Acidity	None	+ Fe	.....	.....	None	.....	.....	.....
Ascorbic acid	9999 : 1	− Fe	.....	.....	None	.....	.....	.....
Total sugars	9999 : 1	+ Fe	None	.....	9999 : 1	+ Fe	None	.....
Reducing "	None	.....	None	.....	9999 : 1	+ Fe	None	.....
Sucrose	9999 : 1	+ Fe	None	.....	None	.....	None	.....
Starch	4000 : 1	+ Fe	9999 : 1	+ Fe	9999 : 1	+ Fe	29 : 1	+ Fe
Pentosans	9999 : 1	+ Fe	None	.....	9999 : 1	+ Fe	None	.....
Hexosans	9999 : 1	− Fe	45 : 1	− Fe	None	.....	43 : 1	+ Fe
Celluloses	None	.....	None	.....	None	.....	None	.....
Lignin	None	.....	None	.....	None	.....	None	.....

* By CONRAD'S formula and from LOVE'S table.

the different treatments were small and lacked statistical significance. These small differences were in favor of the plants of the N-n series. It is suspected that they resulted from differences in the water content of the tissues, as shown in former publications (**10, 11**). The high cellulose content of the transitional subchlorophyllous (2) sections of the leaves of the "mature" (C) and "active" (D) groups is of great interest because it conforms with their structural requirements. The leaves of these groups require, on account of their great length and weight and their outwardly inclined position on the stem, greater structural reinforcement than do the leaves of the other groups in order to prevent their downward collapse.

Cellulose, being a structural and not a food or energy-yielding polysaccharide, did not undergo appreciable changes as a result of changes in the

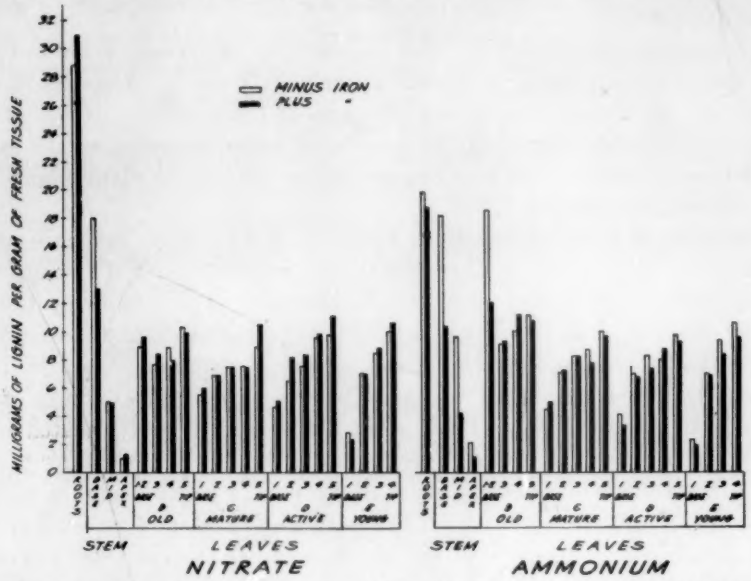


FIG. 16. Lignin in pineapple tissues from plants grown with or without iron, and with nitrate or ammonium nitrogen.

carbohydrate metabolism of the plants induced by the respective amounts of iron of the F and 0 cultures. Except for the smaller weights of the plants of the 0 than F cultures of the N-n series, no other changes could be observed appreciably affecting the values of cellulose.

LIGNIN

The lignin content of the plants of the different treatments recorded in tables V and VI, and in figure 16, ranged from one-half to one-tenth and averaged about one-fourth that of cellulose. Lignin, like cellulose, being a structural and not a food or energy-yielding substance was affected very little by the different amounts of iron in the 0 and F cultures. The data gathered so far indicate that the amounts of lignin, celluloses, and hemicellu-

loses were directly related to the quantities of the fibrovascular tissues of the respective plant sections.

Discussion

The traces of iron contained as impurities in the C. P. nutrient salts were not sufficient to promote good growth in the plants of the 0 cultures of the N-n series where the initial pH of the solution culture changed in a two-week period from 4.4 to 6.8. Such traces of iron were very effective, however, for promoting plant growth in the 0 cultures of the A-n series because the order of pH changes in the solution culture was reversed, shifting in the course of two weeks from an initial pH value of 6.6 to a final one of 4.3. Such changes affected the degree of iron solubility and its availability to plants (**12**). Where iron was available, as in the F cultures, chlorophyll was produced in ample amounts.

The minimum amounts of iron required by *A. comosus* for optimum chlorophyll development and growth are relatively small. Amounts ranging from 0.3 to 0.5 micrograms of iron per gram of fresh tissue have been found in many green tissues and may be considered adequate provided the rate of flow which maintains such iron concentrations within the plant tissues does not diminish appreciably (**12**).

The most obvious physiological function of iron is its influence on the formation of chlorophyll or more probably of protochlorophyll (**4**) and other related metalloporphyrins such as cytochrome-*c*, (**2**). Following the formation of chlorophyll the entire metabolic tempo of the plant is accelerated with respect to carbohydrate synthesis, nitrogen assimilation, and nutrient mineral requirements. This indicates that the physiological effects of iron on the general metabolism of the plant are very intimately related to the chlorophyll status of the plant. Hence it will be utterly misleading to place too much emphasis on the amounts of iron found in plant tissues or on those supplied externally to plants through solution cultures, and too little emphasis on the chlorophyll content of the leaves inasmuch as experience has shown that amounts of chlorophyll in the leaves do not always correlate with those of iron.

Certain interesting phenomena associated with the distribution of ascorbic acid are the gradients of concentrations of this substance in different sections of the leaves of different groups which in most cases were highest in the terminal and lowest in the basal sections. These gradients might have resulted from differences in the physiological functions of the different sections. The data also show that ascorbic acid had reached maximum values in the leaves of the "active" (D) and minimum values either in the "old" (B) or "young" (E) groups. The differences in the amounts of ascorbic acid between groups of leaves may be explained by the assumption that ascorbic acid, being a by-product of metabolic activity (possibly resulting, like many other acids, from the incomplete oxidation of sugars during respiration), is produced in greater amounts in leaves of great vigor and maximum synthetic activity such as the "active" (D) group than in immature

leaves such as the "young" (E) group. The relatively small amounts of ascorbic acid in the leaves of the "old" (B), and to a lesser extent of the "mature" (C) group, resulted possibly from catabolic processes caused by tissue senility. The lower values of ascorbic acid in the F than 0 cultures suggest possible oxidation of ascorbic acid by the iron of F cultures in all the groups of leaves of the N-n series [where average total Fe values, reported elsewhere (**12**), were 19.4 and 47.3 γ per gram of fresh leaf tissue for the 0 and F cultures, respectively]. In the "old" (B) and "mature" (C) leaf groups of the A-n series reduction in ascorbic acid values might have also been caused by oxidation since iron was 3.14 times more abundant in the F than 0 plants (F = 98.5, and 0 = 31.4 γ per gram of tissue). The only exceptions were certain sections of the leaves of the "active" (D) and "young" (E) groups of the A-n series, where the amounts of both ascorbic acid and iron were higher in the F than 0 cultures.

The values of titrable acidity, pH, and ascorbic acid indicate that the conditions in the chlorophyllous sections of the leaves were highly favorable for the solubility and reduction of iron, whereas in the basal sections of the leaves and in the stem they were not as satisfactory. However, chemical analyses for iron, reported previously (**12**), have shown no accumulations of iron either in the stem or in the basal sections of the leaves.

The plants of the N-n series, although including smaller amounts of chlorophyll than those of the A-n series of comparable iron cultures, contained greater amounts of sugars, suggesting that the conditions which had affected the metabolism of nitrogen rather than the amounts of chlorophyll were responsible for these differences.

The distribution of sugars varied considerably in the different groups of leaves. Such variations were caused by differences in the physiological functions of the sections and by the relative degree of vigor of the different groups of leaves. An analysis of such functions shows that the basal sections (1), lacking in chlorophyll, play no part in the photosynthetic processes of the plant in contrast to the chlorophyllous sections (2, 3, 4, 5) which are intimately associated with this mechanism. Also, the terminal sections (5) become senile and lose a great deal of their vigor and photosynthetic efficiency sooner than the immediately adjoining sections (2, 3, 4). Losses of vigor and photosynthetic activity are greater in the leaves of the "old" (B) and "mature" (C) than of the "active" (D) and "young" (E) groups. Therefore, accumulations of sugars in any section of the leaves should be associated either with a decreased rate of utilization as in cases of retarded growth, with highly favorable conditions for photosynthetic activity, or with a decreased rate of translocation from the leaves to the other organs. Low sugar values should be associated with conditions quite opposite those favoring accumulation. Thus, the lower values of sugars in the basal (1) sections of the leaves of the "young" (E) than of the "active" (D) or "mature" (C) groups suggest, on the basis of the above postulations, that a greater rate of sugar utilization for the building of new tissues was in

effect in the "young" (E) than in the "active" (D) or "mature" (C) and "old" (B) groups of leaves. Also, the accumulation of sugars in the terminal sections (5) of the leaves of all such groups may be attributed, on the basis of the same postulations, to a retarded rate of translocation or to the synthesis of much greater amounts than required for the energy-consuming processes of the cells and for the synthesis of various substances. However, certain exceptions to the above postulations as, for example, the slightly greater amounts of sugars in the basal sections of the leaves of the "old" (B) group of the 0 than F cultures of the N-n series cannot be satisfactorily interpreted. It is possible, however, that the higher values of sugars in the basal sections may be attributed either to cessation of the enzymic processes responsible for starch synthesis or to opposite effects such as hydrolysis of reserve starches resulting from tissue senility. The low sugar values in the medial section of the stem may be attributed to the rapid conversion of sugars into starch by very active enzymatic processes. The preponderance of dextrose and the relative absence of levulose in the stem might have resulted from hydrolyzed maltose which probably could have been released from starch by hydrolysis.

The amounts of sucrose increased gradually from the basal to the apical sections of the leaves and stem except in the transitional subchlorophyllous sections (2) of the leaves of the "young" (E) group where they decreased because of a high rate of metabolic activity tending to prevent the accumulation of this or other sugars in appreciable amounts.

Starch, although a reserve product like sucrose, accumulated in greater quantities in the stem than in the leaves. The stems of the N-n series contained almost twice as much starch as those of the A-n series. It is possible that this condition resulted either from differences in the rate of the photosynthetic activity of the leaves of the two series, or from the energy relations involved in the assimilation of the two forms of nutrient nitrogen. There is at present some suspicion, based on evidence from studies on the toxicity of chlorides to *A. comosus* (**13**), that chlorides supplied as $CaCl_2$ to nutrient solutions of the A-n series interfered with the polymerization of sugars into starches in the stem.

The small differences between the amounts of hemicelluloses in the plants of the F and 0 cultures indicate that they play a relatively minor rôle as energy-yielding substances in the carbohydrate economy of *A. comosus*. The data show that the hemicellulose content of the tissues was increased more by nitrate-nitrogen than by the amounts of iron in the F and 0 cultures.

The association of high values of hemicellulose sugars with plant organs containing great amounts of fibrovascular materials [such as the roots and to some extent the basal and terminal sections of the leaves of the "old" (B) and "mature" (C) groups] suggests the possible participation of these substances in the structural formation or cementation processes of fibers.

The amounts of celluloses and lignins were affected very little by the differences in the amounts of iron and by the forms of nitrogen. Both cellu-

lose and lignin, representing structural units in the plant body, remained in a static condition and were not susceptible to changes which had affected such energy-yielding carbohydrates as sugars and starches. The effects of the different treatments on cellulose and lignin were about the same as those on hemicelluloses, but differed considerably from those on starches or sugars.

Summary

The effects of plus- or minus-iron cultures in association with nitrate- or ammonium-nitrogen were studied in connection with the distribution of chlorophyll, carotenoids, ascorbic acid, sugars, starches, hemicelluloses, celluloses, and lignin in different sections of the leaves, stem, and roots of *A. comosus* and are summarized as follows.

1. Chlorophyll and carotenoid pigments were higher in the leaves of the plus-iron than minus-iron cultures. The plants of the ammonium-nitrogen series contained more pigments than the nitrate-nitrogen series for comparable iron cultures which possibly resulted from a greater availability of iron in the former than in the latter series.

2. Titrable acidity gradients reported as citric acid, increased from the basal to the terminal sections of the leaves of the plants of all cultures. The pH values of the sap were approximately inversely proportional to those of titrable acidity. The stem and roots had low values of titrable acidity and showed either little or no changes in the different treatments. The high acidity values of the sap from the chlorophyllous sections of the leaves and the exceedingly small amounts of iron present in the same tissues preclude the possibility of iron precipitation in these tissues of this organ.

3. Ascorbic acid was almost limited to the chlorophyllous sections of the leaves although the amounts present were not directly proportional to those of chlorophyll in the plants of the different cultures. The non-chlorophyllous sections of the plant, including the basal leaf, the stem, and the roots, contained either none or only mere traces of ascorbic acid. The gradients of distribution of ascorbic acid in the chlorophyllous sections of the leaves suggest that certain phases of metabolism rather than amounts of chlorophyll in these tissues were responsible for their ascorbic acid content. Reduction of the ascorbic acid values in old and senile leaves of plus-iron cultures suggests its possible oxidation by iron.

4. Sugars were found in greater amounts in the leaves of the "old," "mature," and "active" groups of the plus-iron than minus-iron cultures. They were higher in the plants of the nitrate- than of the ammonium-nitrogen series. The source of nitrogen in the nutrient solutions had influenced the amounts of sugars more than the amounts of chlorophyll in the leaves. Accumulations of sugars were greater in the basal sections of leaves of the "mature" than "young" groups because the rate of their utilization for new tissue-building processes had decreased in the former. Sugar values in the basal, medial, and apical sections of the stem were in most cases indirectly proportional to those of starches, suggesting that the rate of withdrawal of sugars from the stem is directly proportional to that of starch synthesis.

5. The amounts of levulose were slightly higher in the leaves of the minus-iron than plus-iron cultures of the nitrate-nitrogen series. Levulose was almost absent in the stem. Dextrose, occurring in amounts twice as great or more than levulose, was distributed in all organs of the plant. Sucrose was higher in the leaves of the plus-iron than minus-iron plants of the nitrate series, but in the ammonium series it was as high or slightly higher in the minus-iron than in the plus-iron cultures.

6. Starch was consistently higher in the stem of the plus- than minus-iron cultures, the former containing 101.3 per cent. more starch for the ammonium series and 33.2 per cent. more for the nitrate series. The plants of the nitrate-nitrogen series contained 161.6 per cent. more starch for the plus-iron and 295.0 per cent. for the minus-iron cultures than of the ammonium-nitrogen series.

7. The differences in total hemicellulose sugars between the plants of the plus- and minus-iron cultures were slight, with the plants of the former cultures containing, in most sections, greater amounts than the latter. The plants of the nitrate-nitrogen series contained greater amounts of hemicellulose sugars than those of the ammonium-nitrogen series. The differences between the amounts of hemicelluloses in the plants of the nitrate- versus the ammonium-nitrogen series were greater than those between the plus- versus the minus-iron cultures. The pentosan fractions of hemicelluloses of the nitrate series were higher in the plus- than in the minus-iron cultures, while the hexosan fractions were reversed. In the ammonium series the distribution of hexosans throughout the plant was irregular.

8. The amounts of cellulose were higher in the transitional sections which bear the strain for the erect position of the young leaves and reclined position of the mature leaves.

9. Lignin was generally high in those parts of the plant containing great amounts of fibrovascular tissues such as the roots, basal sections of the stem, and terminal sections of the leaves.

10. The amounts of both celluloses and lignin were affected only slightly by the different treatments and behaved in this respect like the hemicelluloses.

PINEAPPLE RESEARCH INSTITUTE
UNIVERSITY OF HAWAII
HONOLULU, T. H.

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Methods of analysis. 1940.
2. BALLS, ERIC G. Biological oxidations and reductions. Ann. Rev. Biochem. **11**: 1–25. 1942. Stanford University Press, California.
3. BESSEY, O. A., and KING, C. G. The distribution of vitamin C in plant and animal tissues and its determination. Jour. Biol. Chem. **103**: 687–698. 1933.

4. EYSTER, W. H. Protochlorophyll. Science n.s. **68**: 569–570. 1928.
5. PETERSON, C. J., WALDE, A. W., and HIXON, R. M. Effect of temperature on sulfuric acid method of lignin. Ind. & Eng. Chem. Anal. Ed. **4**: 216–217. 1932.
6. PUCHER, C. W., and VICKERY, H. B. Determination of starch in plant tissues. Ind. & Eng. Chem. Anal. Ed. **8**: 92–97. 1936.
7. QUISUMBING, F. A., and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved current in methods. Jour. Amer. Chem. Soc. **43**: 1503–1526. 1921.
8. SCHERTZ, F. M. The extraction and separation of chlorophyll (a + b) carotin and xanthophyll in fresh leaves preliminary to their quantitative determination. Plant Physiol. **3**: 211–216. 1928.
9. ————. The quantitative determination of chlorophyll. Plant Physiol. **3**: 323–334. 1928.
10. SIDERIS, C. P., KRAUSS, B. H., and YOUNG, H. Y. Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. **13**: 489–527. 1938.
11. ————, ————, and ————. Distribution of different nitrogen fractions, sugars and other substances in various sections of the pineapple plant grown in soil cultures and receiving either ammonium or nitrate salts. Plant Physiol. **14**: 227–254. 1939.
12. ————, YOUNG, H. Y., and KRAUSS, B. H. Effects of iron on certain physiochemical properties and ash constituents of *Ananas comosus* (L.) Merr., grown in solution cultures supplied either with nitrate or ammonium salts as sources for nitrogen. Plant Physiol. **18**: 608–632. 1943.
13. ————, and YOUNG, H. Y. Influence of potassium on chloride toxicity in *Ananas comosus* (L.) Merr. Amer. Jour. Bot. **27**: 21s. 1941.
14. WIDDOWSON, ELSIE MAY. A method for the determination of small quantities of mixed reducing sugars and its application to the estimation of the products of hydrolysis of starch by taka diastase. Biochem. Jour. **25**: 863–879. 1931.

RAPID ANALYTICAL METHODS FOR SOME OF THE MORE COMMON INORGANIC CONSTITUENTS OF PLANT TISSUES

R. C. LINDNER

(WITH ONE FIGURE)

Introduction

In recent years, data have gradually been accumulated indicating the usefulness of leaf analysis of plants as an aid in the diagnosis of nutritional disorders of crop plants and as a guide in determining the fertilizer program. This technique seems especially important with some plant disorders of a mild nature in which no definite foliage symptoms are produced, yet in which the vigor and yield of the affected plants or the quality of plant products are reduced. LAGATU and MAUME in France (**4**), and THOMAS (**6**), and THOMAS and MACK (**7**) in this country have laid the foundations for leaf analysis or "foliar diagnosis," and an increasing number of other workers are adding to the knowledge of the subject. One of the factors that has delayed the general adoption of leaf-analysis methods is the time-consuming and expensive procedures generally used in analyzing plant material. Another factor is the manner in which the data of leaf analysis has been interpreted. It is the purpose of this paper to present rapid and inexpensive procedures for determining some of the more common inorganic plant nutrients.

Many of the standard methods of analyzing plant material are based on volumetric procedures which are quite accurate but are not suitable for the rapid testing of a number of samples. For diagnostic purposes, accuracy of methods is of secondary importance to speed, for sampling errors are usually very much larger than errors in analysis. Colorimetric methods are readily adapted to diagnostic procedures, for they are rapid, sensitive, and inexpensive. With the development of the photo-electric colorimeter in recent years, this type of analysis has become even more rapid and has attained a fair degree of accuracy.

In most standard analyses, nitrogen is determined on a part of the sample by the regular Kjeldahl procedure. Another portion of the sample is then ashed (in the presence of some substance to prevent the volatilization of phosphorus), and the other elements are determined in the dissolved ash. It is obvious that considerable time could be saved if the organic matter were destroyed in such a way that all of the more common elements (N, P, K, Ca, and Mg) could be determined on the same sample. The following methods were developed with this course of procedure in mind.

Equipment

The only special piece of equipment needed is a photo-electric colorimeter, preferably of the test-tube type. Manual colorimeters can be used, but they

are more time-consuming and less accurate than the photo-electric type. An ample supply of standard colorimeter tubes (at least 12), calibrated at 5 ml. and 10 ml., and an assortment of red, green, and blue filters are necessary. The colorimeter tubes should be standardized before use, for they are very seldom exactly alike.

A fume hood is desirable but not entirely essential. Ample supplies of 50-ml. Erlenmeyer flasks, 50-ml. and 100-ml. glass-stoppered volumetric flasks, and glass-stoppered dropping bottles are desirable, along with an assortment of pipettes and other standard laboratory equipment. In addition, a leaf punch should be available.

Sampling

The sampling is perhaps the most important part of any analytical procedure for determining the constituents of plant material, because it is the source of greatest error. For a sample, either the entire plant itself or some part of the plant that will reflect the nutritional status of the plant as a whole should be used. The leaf is perhaps the best part of most plants for this purpose, but care must be taken to secure leaves of the same physiological age, same type, and same relative position on the plant. Trees offer further complications since the main branches of the tree often act as individual units. For a further discussion of sampling see THOMAS (**6**).

The dry weight of the material is usually used as a basis of comparison in expressing the results of analyses. The use of leaf area as a basis of comparison has certain advantages. A leaf punch which cuts out exactly 1 sq. cm. of leaf tissue may be used, and with this instrument a large number of samples may be collected in a minimum of time. The methods presented here are applicable to either fresh or dry material. If fresh material is used, the samples can be directly analyzed, saving the time required for the drying, grinding, and weighing of dry samples. Ten sq. cm. of fresh leaf material or 100 mg. of dry material is the most convenient size of sample. At times, it may be desirable to have the leaves from which samples are secured remain on the plant, and, by using the leaf punch, 1 sq. cm. of leaf material can be removed from each of ten leaves without removing the leaves from the plant. The leaves of some plants, of course, are not of sufficient size for area sampling (grasses, for instance), and the dry material must be used.

Digestion

The digestion procedure has been discussed elsewhere (**5**) but will be presented here for completeness.

REAGENTS

1. Concentrated sulphuric acid (c.p.—nitrogen free).
2. 30 per cent. hydrogen peroxide (c.p.—nitrogen and preservative free).

PROCEDURE

Transfer about 100 mg. of dry material or 10 sq. cm. of fresh material to a 50-ml. Erlenmeyer flask. Add 2 ml. of concentrated sulphuric acid

and heat gently over a flame in a fume hood until the sample is broken down and partially dissolved. If nitrates are present, continue digestion for about a minute after dense fumes have been given off to allow for complete reduction of the nitrates by the organic matter. Cool and add 0.5 ml. of 30 per cent. hydrogen peroxide. Heat gently—the solution should become clear and colorless. Continue the heating until dense fumes are given off—usually the solution becomes darker at this stage. Cool, and add 5 drops more of 30 per cent. hydrogen peroxide and heat again—repeat this procedure if necessary. No more than 5 drops of hydrogen peroxide should be added at one time after the first addition, because a large excess of peroxide in the absence of organic matter will oxidize some of the ammonia. When the solution is perfectly clear and colorless on continued heating, cool, dilute with water, and transfer with washings to a 100-ml. volumetric flask and make to volume.

Determinations

Nitrogen

The procedure for determination of nitrogen has been discussed elsewhere (**5**) but will be presented here for completeness.

Reagents.—

1. 2.5 N sodium hydroxide.
2. 10 per cent. sodium silicate.
3. Nessler solution.

The Koch-McMeekin Nessler solution (**3**) was used in this work, although others that are more easily prepared would probably be just as satisfactory. This solution is best stored in a large bottle and transferred to a small dropping bottle for use. It is made as follows: "Dissolve 30 grams of KI in 20 ml. of distilled water, and add 22.5 gm. of iodine to the solution. Shake until dissolved, and then add 30 gm. of pure metallic mercury. Shake the mixture well, keeping the solution cool by holding under running tap water from time to time, until the supernatant liquid has lost the yellow color of iodine. Pour off from the undissolved mercury and test for the presence of excess iodine by adding a few drops to starch solution in a test tube (or spot plate). If no blue color is obtained, add iodine solution similar to that described above, drop by drop, until there is a faint excess of free iodine as determined by testing a few drops with starch solution. Dilute to 200 ml., mix, and pour into 975 ml. of accurately prepared 10 per cent. sodium hydroxide solution. Mix well and allow any precipitate to settle out, using the clear supernatant fluid. Avoid stirring up the sediment when removing from the storage bottle."

Procedure.—Transfer a 10-ml. aliquot of the peroxide-digested material to a 50-ml. volumetric flask. Add 2 ml. of 2.5 N sodium hydroxide (conveniently added from a small burette) to partially neutralize the excess acid, and 1 ml. of 10 per cent. sodium silicate to prevent turbidity. Make to volume and mix well. Transfer a 5-ml. aliquot to a colorimeter tube and add 4 drops of Nessler's reagent, mixing thoroughly after the addition of

each drop. If the mixing is not thorough, additional drops of reagent will be required to obtain the maximum color. Allow to stand for several minutes before taking a colorimeter reading. A blue filter (such as Wratten no. 49) should be used.

REMARKS.—Beer's law is not followed, and a curve must be prepared from readings based upon standard solutions of ammonium sulphate. Individual colorimeters and working conditions vary so much that standard curves should be prepared for each instrument. If a 100-mg. sample is used, the range of nitrogen, in percentage of the dry weight, is from about 0.01 per cent. up to about 4.00 per cent. Above about 2.00 per cent., the sensitivity gradually decreases. The success of this method depends upon the use of sodium silicate to prevent the formation of a turbidity when the Nessler solution is added.

PHOSPHORUS

The method for phosphorus determination is an adaptation of standard colorimetric methods based upon the formation of molybdenum blue.

REAGENTS.—

1. 2.5 N sodium hydroxide.
2. Molybdate reagent.
   2.5 per cent. ammonium molybdate in 28 per cent. sulphuric acid.
3. Stannous chloride.
   2.5 gm. $SnCl_2 \cdot H_2O$.
   10 ml. conc. HCl.
   90 ml. H_2O.
   This solution should be prepared fresh for use. It will keep for about a week in a glass-stoppered bottle.

PROCEDURE.—Transfer a 10-ml. aliquot of the peroxide-digested material to a 50-ml. volumetric flask. Add 2.5 ml. of 2.5 N NaOH to neutralize the excess acid, and then add 2 ml. of the molybdate reagent. Make to volume and add 2 drops of stannous chloride solution. Mix well and let stand for several minutes to allow for maximum development of the blue color. Take a colorimeter reading on a 5-ml. aliquot using a green filter (such as Wratten no. 54). The color reaches a maximum in about 2 minutes after adding stannous chloride but begins to fade after 10 minutes, so that a reading should be taken during this interval.

REMARKS.—If a 100-mg. sample is used, the range of phosphorus in percentage of the dry weight is from about 0.004 per cent. to about 0.400 per cent. Beer's law is valid in this range. For larger amounts of phosphorus, a greater dilution should be used. For smaller amounts, a red filter, which increases the sensitivity about 4 times, may be used. A blank determination on all reagents should be run precisely as above.

The hydrogen ion concentration should be controlled within fairly narrow limits. Before adding the molybdate reagent, the sample should be about 0.06 N in acid.

Although arsenates interfere with the method, there usually is not enough arsenic present to significantly affect the results. Spray residues containing arsenic should be removed from the leaf before it is sampled. If arsenates are known to be present in appreciable amounts, they should be reduced to arsenites, which do not interfere. This can be done by adding 0.5 gm. of sodium bisulphite to a 10-ml. aliquot of the peroxide-digested material in a 50-ml. volumetric flask. Place in an oven at 80° C. for 30 minutes and then cool rapidly under the tap and proceed as above. The excess bisulphite does not interfere.

Potassium

The method for determination of potassium is a turbidimetric method based upon the precipitation of potassium as the cobaltinitrite in a finely suspended form.

Reagents.—

1. Methyl red indicator.
   100 mg. methyl red in 100 ml. of 50 per cent. alcohol.
2. Saturated sodium carbonate solution.
3. 6 N sulphuric acid.
4. Sodium nitrate (granular—reagent grade—K free).
5. 95 per cent. ethyl alcohol.
6. Sodium cobaltinitrite (reagent—powder).

Procedure.—Transfer a 5-ml. aliquot of the peroxide-digested material to a 50-ml. Erlenmeyer flask. Add one drop of methyl red indicator, and neutralize the solution by the drop-wise addition of a saturated solution of sodium carbonate. Add two drops excess of sodium carbonate, and boil the solution vigorously for several minutes to expel the ammonia. Cool, and carefully transfer with a minimum of washings to a colorimeter tube; the sample plus the washings should not amount to more than 6 ml. Neutralize the excess sodium carbonate with about 2 drops of 6 N sulphuric acid to about pH 6. Add about 0.5 gm. of sodium nitrate crystals and shake to dissolve. Then add 4 ml. of 95 per cent. alcohol, mix, and make to 10-ml. mark with water. The mixing is most easily done with a small glass rod flattened on the end. Carefully add about 0.17 gm. of dry powdered sodium cobaltinitrite with a small porcelain spoon. The exact amount used is not important as long as the same amount is used each time. As soon as the reagent is added, the solution should be mixed thoroughly with the aid of the glass rod. Allow to stand for ten minutes before taking a reading on the colorimeter, using a red filter.

Remarks.—A blank determination must be made on all reagents precisely as above. If a 100-mg. sample is used, the range of potassium in percentage of the dry weight is from about 0.02 per cent. to about 6.00 per cent. There is a straight-line relationship between the concentration of potassium and the turbidity in this range. The success of the method depends upon the exact duplication of the procedure every time it is used.

The concentration of salts is important as they influence the size of the precipitate particles. It is important to use exactly 4 ml. of alcohol. When larger amounts are used, the salts are precipitated, and when smaller amounts are used, the potassium is not completely precipitated. Ammonium ions interfere with the method since they give the same precipitate with cobaltinitrite as does potassium. Ammonia is easily removed by boiling in alkaline solution; completeness of removal may be checked by the odor or with a moist piece of indicator paper held over the mouth of the flask. Sodium hydroxide is not satisfactory for neutralizing the solution since most grades are apt to contain appreciable amounts of potassium. Readings on the colorimeter are usually constant for a period of several hours. Larger amounts of potassium tend to settle out after about 15 minutes, but agitation of the mixture will bring it back to the original state of dispersion. Before taking a colorimeter reading, all entrapped air bubbles in the solution should be allowed to rise.

Calcium

The method for determination of calcium is a turbidimetric method based upon the precipitation of calcium as the oxalate in a finely suspended state.

Reagents.—

1. Sodium carbonate (dry powdered reagent).
2. Ammonium oxalate (crystalline reagent).
3. Gum acacia reagent (the gum acacia should be purified before use as the commercial material contains appreciable amounts of calcium and magnesium salts).

Dissolve 10 gm. of commercial gum acacia in about 100 ml. of water. Add about 0.6 gm. of ammonium oxalate crystals and centrifuge to remove the precipitate that forms. Decant the supernatant liquid, acidify it with about 10 ml. of 6 N HCl, and add ethyl alcohol until a dense white flocculent precipitate of arabic acid forms. Add sufficient alcohol to precipitate all of the arabic acid. Allow to stand for several minutes to permit the precipitate to settle and discard the supernatant liquid. Wash the precipitate several times with 95 per cent. alcohol, allowing it to settle after each washing. Dissolve the precipitate in about 100 ml. of water, add 10 ml. of 6 N HCl and again precipitate with alcohol. Wash twice with alcohol and finally take up the arabic acid in about 200 ml. of water. Add about 60 gm. of sodium acetate, and when this is in solution, centrifuge to remove any extraneous matter.

Procedure.—Transfer a 5-ml. aliquot of the peroxide-digested material to a colorimeter tube and partially neutralize with about 0.12 gm. of sodium carbonate powder, using an appropriate sized spoon. Add 1 ml. of gum acacia reagent and shake the solution thoroughly. Take a reading at this time on the colorimeter for a reagent blank. Then add about 0.1 gm. of powdered ammonium oxalate with an appropriate sized spoon and shake the mixture thoroughly. Allow to stand for ten minutes before taking a reading on the colorimeter, to permit complete precipitation of the calcium. The

tube should be shaken vigorously immediately before a reading is taken so as to bring any precipitated material into suspension. Use a green filter (such as Wratten no. 54).

REMARKS.—Beer's law is not followed, and a standard curve must be plotted using known amounts of calcium under the exact conditions that will be found in the unknown sample. The method is not accurate for very small amounts of calcium (below 1.00 per cent. Ca when using 100-mg. samples) due to the solubility of the calcium oxalate. If very small amounts of calcium are present, it may be desirable to add a known amount of calcium to bring the reading up to the more sensitive part of the curve. In this case 1 ml. of a standard calcium solution and 4 ml. of the unknown can be used, and the reading corrected for the added calcium. It is important to follow the procedure carefully as small changes may tend to affect the results appreciably. The colorimeter tube must be shaken vigorously immediately after the addition of ammonium oxalate to obtain a uniform dispersion of the calcium oxalate. Magnesium does not interfere so long as it is present in lower concentrations than is calcium; a condition which holds true for most plant material. The addition of sodium acetate to the gum acacia reagent buffers the solution to the proper pH for precipitation of the calcium oxalate. It is important to allow the colorimeter tubes to stand ten minutes before taking readings to allow for complete precipitation of the calcium. Longer standing, up to several hours, does no harm as long as the tubes are shaken vigorously immediately before readings are taken to re-suspend any material that may have settled out. Consistent results are not obtained in the absence of gum acacia; this material is necessary to maintain uniform particle size.

MAGNESIUM

The method for determination of magnesium is a modification of the colorimetric titan yellow (thiazol yellow) procedure of GILLAM (**2**).

REAGENTS.—

1. 4 per cent. aqueous solution of hydroxylamine hydrochloride.
2. Titan yellow solution.

   0.15 gram of the dye dissolved in 75 ml. of ethyl alcohol and 25 ml. of water. Filter before using.
3. 40 per cent. sodium hydroxide solution.

PROCEDURE.—Transfer a 5-ml. aliquot of the peroxide-digested material to a colorimeter tube. Add one ml. of hydroxylamine solution and ten drops of titan yellow. Mix the solution well and add 1 ml. of 40 per cent. sodium hydroxide. After thorough mixing, cool the tube to 20° C. in a water bath and take a reading immediately in the colorimeter, using a green filter (such as Wratten no. 54).

REMARKS.—If a 100-mg. sample is used, the range of magnesium in percentage of dry weight is from about 0.003 per cent. to about 0.750 per cent. Beer's law is followed in this range; but in higher concentrations the

magnesium tends to settle out. There is a rather high reagent blank reading and it is important to determine this carefully under the same conditions to be used for the samples. Since there are certain variable factors inherent in the method, at least three determinations should be averaged on each sample. It is essential to have the tubes at some standard temperature before taking readings on the colorimeter. The exact temperature is immaterial, but it is more convenient to standardize the method near room temperature. About five minutes in the water bath is usually sufficient to bring the tubes to the proper temperature.

The hydroxyl-ion concentration should be kept within fairly narrow limits. In general, the higher the hydroxyl-ion concentration, the higher the reading. If a smaller aliquot than 5 ml. is used, it should be made up to 5 ml. in the colorimeter tube with 2 per cent. sulphuric acid so as to keep the original acid concentration about the same.

Calcium, phosphorus, and iron interfere with this method, but, fortunately, these substances are usually not present in plant material in high enough concentration to appreciably influence the results. For example, in order to interfere, the calcium concentration must be at least 100 times that of the magnesium, and the phosphorus concentration at least 20 times that of the magnesium.

Discussion

Nitrogen

A sufficient discussion of the nitrogen method has been presented elsewhere (**5**) and need not be repeated here.

Phosphorus

In table I is presented a comparison of phosphorus determination by the rapid method with those obtained by the official A.O.A.C. micro method (**1**) on samples of dry apple leaves. In the official method, 1-gm. samples of leaf material were used, while in the rapid method 100-mg. samples were

TABLE I

Comparison of rapid method for phosphorus with the official micro-method on samples of dry apple leaves

Sample	Phosphorus as percentage of dry weight					
	Official micro-method			Rapid method		
61	0.313			0.308		
	0.310	0.311	average	0.312	0.311	average
	0.311			0.312		
111	0.272			0.268		
	0.268	0.269	"	0.268	0.269	"
	0.267			0.271		
B–26	0.117			0.120		
	0.117	0.118	"	0.120	0.119	"
	0.119			0.117		

TABLE II

RECOVERY OF POTASSIUM FROM PURE SOLUTIONS OF POTASSIUM CHLORIDE

MICROGRAMS OF POTASSIUM TAKEN	COLORIMETER READING	MICROGRAMS OF POTASSIUM RECOVERED	PERCENTAGE RECOVERY
0	63	.....	.....
	64	.....	.....
	70 av. 65	.....	.....
	64	.....	.....
	63	.....	.....
25	89	23	92
	94	27	108
	90	24	96
	92	25	100
	93	26	104
50	120	52	104
	120	52	104
	115	47	94
	116	48	96
	122	54	108
100	170	99	99
	176	104	102
	166	95	95
	168	97	97
	171	100	100
200	278	200	100
	274	196	98
	282	204	102
	277	199	99.5
	280	202	101

used. It can readily be seen that the two methods are comparable in accuracy. This should be expected as the methods are very similar and the same photo-electric colorimeter was used in both.

POTASSIUM

The recovery of potassium from pure potassium chloride solutions is shown in table II. There are certain variations in any group of individual

TABLE III

COMPARISON OF THE RAPID METHOD FOR POTASSIUM DETERMINATION WITH A VOLUMETRIC COBALTINITRITE METHOD ON DRY APPLE LEAVES

SAMPLE	POTASSIUM AS PERCENTAGE OF DRY WEIGHT	
	VOLUMETRIC METHOD	RAPID METHOD
	%	%
a–67	1.51	1.49
	1.50	1.54
	1.51	1.52
a–74	3.81	3.90
	3.84	3.80
	3.82	3.82
a–76	0.87	0.85
	0.87	0.90
	0.86	0.86

readings, but the average of at least three determinations is usually fairly accurate. The blank reading is due to the color of the reagents.

In table III is shown a comparison between the rapid turbidimetric method and a volumetric method (unpublished). In the volumetric method the potassium is precipitated as the cobaltinitrite, and the supernatant solution is filtered off by means of a filter stick. After the precipitate is washed, it is dissolved with an excess of ceric sulphate, and the mixture is titrated with ferrous ammonium sulphate. 1-gm. samples of dry apple leaf material were used for the volumetric method while 100-mg. samples were used for the rapid method. There is a close agreement between these two methods when used on leaf material.

TABLE IV

RECOVERY OF POTASSIUM ADDED TO DRY APPLE LEAF MATERIAL

POTASSIUM ADDED	TOTAL POTASSIUM FOUND	POTASSIUM RECOVERED	PERCENTAGE RECOVERY
% of dry weight	*% of dry weight*	*% of dry weight*	*%*
0	1.53	.....	.....
	1.50 av. 1.51	.....	.....
	1.49	.....	.....
0.50	2.00	0.49	98
	2.05	0.54	108
	1.98	0.47	94
1.00	2.52	1.01	101
	2.56	1.05	105
	2.50	0.99	99
1.50	2.98	1.47	98
	3.05	1.54	103
	3.00	1.49	99

Potassium can readily be recovered when it is added to dry apple leaf material, as shown in table IV. The potassium was added as potassium chloride to 100 mg. of dry leaf material and evaporated to dryness before digestion.

CALCIUM

The type of standardization curve that can be expected with calcium is shown in figure 1. As was mentioned before, there will probably be certain variations in colorimeters and in conditions of analysis, so that this curve should not be expected to be duplicated exactly under other conditions. With smaller concentrations of calcium, the sensitivity is decreased, probably due to the solubility of the calcium oxalate precipitate. With higher concentrations of calcium, the sensitivity again decreases, probably owing to the formation of larger calcium oxalate particles. By preparing such a curve, under the exact conditions to be used in analysis, fairly good results should be obtained.

Table V shows a comparison of the rapid calcium method with a volumetric micro-method on dry apple leaf material. For the volumetric method

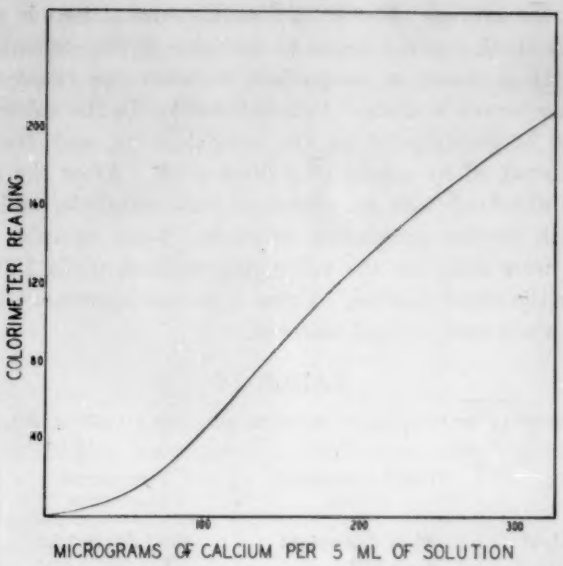


FIG. 1. Type of standardization curve to be expected in calcium determination.

1-gm. samples were used while for the rapid method 100-mg. samples were used. In the volumetric method, the calcium was precipitated as the oxalate and the supernatant liquid removed by means of a filter stick. After washing, the calcium oxalate precipitate was dissolved with an excess of ceric sulphate and the mixture titrated with ferrous ammonium sulphate. Very good agreement was obtained between these two methods.

Table VI shows that calcium is satisfactorily recovered when added to dry apple leaf tissue. The calcium was added as a standard calcium sulphate solution prepared from calcium carbonate and sulphuric acid. The mixture of calcium solution and leaf tissue was evaporated to dryness before digestion.

TABLE V

COMPARISON OF THE RAPID CALCIUM METHOD WITH A VOLUMETRIC MICRO-METHOD ON DRY APPLE LEAF MATERIAL

SAMPLE	CALCIUM AS PERCENTAGE OF DRY WEIGHT	
	VOLUMETRIC METHOD	RAPID METHOD
	%	%
a–76	1.12	1.20
	1.12	1.12
	1.11	1.18
a–134	2.23	2.20
	2.20	2.26
	2.23	2.26
a–132	3.32	3.20
	3.31	3.28
	3.30	3.36

TABLE VI

RECOVERY OF CALCIUM ADDED TO DRY APPLE LEAF MATERIAL

CALCIUM ADDED	TOTAL CALCIUM FOUND	CALCIUM RECOVERED	PERCENTAGE RECOVERY
% of dry weight	*% of dry weight*	*% of dry weight*	*%*
0.0	1.26	.....	.....
	1.26 av. 1.27	.....	.....
	1.30	.....	.....
0.50	1.74	0.47	94
	1.82	0.55	110
	1.78	0.51	102
1.00	2.22	0.95	95
	2.30	1.03	103
	2.26	0.99	99
2.00	3.30	2.03	102
	3.20	1.93	96
	3.20	1.93	96

MAGNESIUM

There are certain errors inherent in the titan yellow method for magnesium, but by following the procedures as outlined, satisfactory results

TABLE VII

RECOVERY OF MAGNESIUM FROM PURE MAGNESIUM SULPHATE SOLUTIONS

MAGNESIUM TAKEN	COLORIMETER READING	MAGNESIUM RECOVERED	PERCENTAGE RECOVERY
micrograms		*micrograms*	*%*
0.0	160	.....	.....
	169	.....	.....
	167 av. 165	.....	.....
	165	.....	.....
	164	.....	.....
5.7	206	5.4	93
	214	6.4	112
	210	5.9	104
	209	5.7	100
	209	5.7	100
	208	5.6	98
11.4	256	11.9	104
	250	11.1	97
	246	10.6	93
	252	11.4	100
	256	11.9	104
	253	11.5	101
17.1	295	17.0	98
	292	16.6	97
	294	16.9	99
	300	17.6	103
	308	18.7	109
	297	17.2	101
22.8	330	21.4	94
	338	22.5	99
	342	23.0	101
	339	22.6	99
	329	21.3	93
	345	23.4	103

TABLE VIII

RECOVERY OF MAGNESIUM ADDED TO DRY APPLE LEAF MATERIAL

MAGNESIUM ADDED	TOTAL MAGNESIUM FOUND	MAGNESIUM RECOVERED	PERCENTAGE RECOVERY
% of dry weight	*% of dry weight*	*% of dry weight*	*%*
0.0	0.313	.....	.....
	0.334	.....	.....
	0.308 av. 0.324	.....	.....
	0.324	.....	.....
	0.339	.....	.....
0.100	0.420	0.096	96.0
	0.446	0.122	122.0
	0.412	0.088	88.0
	0.428	0.104	104.0
	0.428	0.104	104.0
0.500	0.836	0.512	102.4
	0.810	0.486	97.2
	0.822	0.498	99.6
	0.783	0.459	91.8
	0.796	0.472	94.4

may be expected. In table VII some typical data are presented on the recovery of magnesium from pure magnesium sulphate solutions. There is a large blank reading due to the color of the reagents, but if enough readings are taken, the differences are averaged out.

Table VIII shows the recovery of magnesium added to dry apple leaf material. The magnesium was added as a standard solution of magnesium sulphate and the mixture was evaporated to dryness before digestion.

TIME INVOLVED

Table IX shows the approximate time required to perform the procedures reported here. The estimates are based on the length of time taken by an analyst with one assistant to analyze 12 samples of leaf material sampled on an area basis. The approximate time required was six hours, an average of 30 minutes per sample for a complete analysis, including sampling and digestion.

TABLE IX

TIME REQUIRED BY AN ANALYST WITH ONE ASSISTANT TO COMPLETELY ANALYZE TWELVE LEAF SAMPLES

PROCEDURE	TIME FOR 12 SAMPLES
	min.
Sample preparation	30
Digestion	55
Nitrogen	40
Phosphorus	45
Potassium	70
Calcium	40
Magnesium	60

Summary

Rapid colorimetric procedures for the determination of nitrogen, phosphorus, and magnesium, and turbidimetric procedures for the determination of potassium and calcium in the same sample of plant tissues are presented.

BUREAU PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING
AGRICULTURAL RESEARCH ADMINISTRATION
U. S. DEPARTMENT OF AGRICULTURE
WENATCHEE, WASHINGTON

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. Fifth ed. 1940.
2. GILLAM, SHERMAN W. A photometric method for the determination of magnesium. Ind. & Eng. Chem. Anal. Ed. **13**: 499–501. 1941.
3. KOCH, F. C., and MCMEEKIN, T. L. New direct nesslerization micro-Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia. Jour. Amer. Chem. Soc. **46**: 2066–2069. 1924.
4. LAGATU, H., and MAUME, L. Le diagnostic foliare de la pomme de terre. Ann. École. Nat. Agr. Montpellier. Premier memoire **20**: 219–281. 1930.
5. LINDNER, R. C., and HARLEY, C. P. A rapid method for the determination of nitrogen in plant tissue. Science n.s. **96**: 565–566. 1942.
6. THOMAS, WALTER. Foliar diagnosis: principles and practice. Plant Physiol. **12**: 571–599. 1937.
7. ————, and MACK, WARREN B. Foliar diagnosis in relation to soil heterogeneity. Soil Sci. **52**: 455–468. 1941.

EVALUATION OF FOLIAGE INJURY AND WATER LOSS IN CONNECTION WITH USE OF WAX AND OIL EMULSIONS[1]

C. L. Comar and C. G. Barr

(with four figures)

Introduction

The evaluation of the effects of spray materials on plants has been confused by the lack of reliable methods for the measurement of the injury which may be caused by such treatment. The use of wax emulsions for the prevention of desiccation in nursery stock and the application of spray materials for the control of fungous diseases and insect pests are practices which have created numerous problems, many of which have not been satisfactorily solved because of lack of information on the underlying principles involved. This situation has been further aggravated by the continual appearance of new materials which are being developed for these purposes. It has long been recognized that the application of a spray material to a plant may alter the course of photosynthesis, respiration, and transpiration or may result in direct foliage injury.

It is the purpose of this paper to describe methods and equipment which have been used at this station for the evaluation of the effects of spray materials on plants in connection with the development of emulsions to be employed for the prevention of desiccation. Illustrative results are given which indicate the interrelationships existing between chemical composition of the emulsion, toxicity, environmental conditions, and efficiency in protection against excessive water losses.

Transpiration as a factor in plant growth and crop production has perhaps been the subject of more investigations than any other physiological process. Detailed discussions of the water relations of plants may be found in the literature (**4, 5, 6, 11, 13, 16, 18, 20**) and it is evident that while various rôles have been assigned to transpiration, its function has never been clearly and completely explained. Furthermore, there seems to be no agreement as to whether the benefits of the process exceed the ill effects. It cannot be denied, however, that certain cases exist where the ill effects of transpiration seem to far outweigh the apparent benefits. For example, it is often necessary to transplant shrubs and trees when they are in the active or non-dormant stage; cut branches of plants are used for decorative purposes, and the use of transplantings and cut branches in camouflage work is becoming of more importance. With but a few exceptions every section of the country is subject to varying periods of drought. Transpiration in a

[1] This research was supported by the Horace H. Rackham Research Endowment of the Michigan State College of Agriculture and Applied Science for studies on the industrial utilization of agricultural products. Published with the permission of the Director of the Experiment Station as Journal Article no. 658 (n.s.).

genotype is dependent largely on two factors: (a) the saturation deficit or water absorbing power of the atmosphere and (b) the available water. It is evident, then, that if for any reason water cannot be supplied to the leaves as rapidly as it is lost from them, wilting will occur which may result in injury to the plant. The supply of water to the leaves may be limited by a lack of water in the soil or, in certain cases, by an inadequate water transporting mechanism in the plant. In the present paper no consideration has been given to the water relations of diseased plants in which the xylem vessels have been plugged or destroyed by fungi and bacteria.

Following the work of NEILSON (**23, 24**) and others (**19, 22, 25**), melted wax was used with considerable success for the protection of nursery stock in storage, shipment, and transplanting. The difficulties involved, however, in this type of treatment precluded the possibility of the extensive use of melted wax. EMERSON and HILDRETH (**15**) have published a preliminary report on the reduction of transpiration of transplanted evergreens by the use of various materials. The development in 1930 of satisfactory emulsifying agents for paraffin wax (**3, 26**) made possible the production of wax-in-water emulsions which greatly facilitated the application of waxy coverings to plant material. At that time a wax emulsion was developed at this station for spraying nursery stock and other plant material and was later put into commercial production. Later, MILLER, NIELSON, and BANDEMER (**21**) published a bulletin describing the use of this emulsion on dormant ornamental deciduous and coniferous trees and shrubs. The extensive use of this material in recent years for a great variety of purposes revealed its tendency to cause direct foliage injury under certain circumstances. The reasons for the conflicting observations from field tests during the past years were not well understood. This led to the present research which was then concerned with the determination and elimination of the cause of the injury and the possibility of developing a completely non-toxic spray which could be applied to foliage for protection against excessive water loss without causing material damage.

In order to obtain useful results a program of this type must be well rounded and designed so as to effectively treat a wide range of problems varying from the purely chemical to those of a biological nature. The work on this project was therefore planned to provide for: (a) physico-chemical studies concerned with the actual development of the spray emulsion formulas together with investigations of the physical characteristics of the emulsions and the films formed from them; and (b) biological studies in connection with the performance of the spray as conditioned by the characteristics of the plant and the environmental factors. The present paper deals primarily with the biological aspects of the problem.

Experimentation

CONTROLLED ENVIRONMENT CHAMBER

It is not practicable to study in detail the effects of spray emulsions on plants in the field or in the greenhouse because of the many variables in-

volved and the difficulties in reproducing conditions throughout the experiments. After development of satisfactory formulas, extended field tests will serve, of course, as criteria in regard to the usefulness of the emulsions. In order to eliminate the uncertainties of weather conditions, variations in temperature, humidity and light intensity, etc., it was felt that an environment should be available in which these factors could be controlled and varied at will. A controlled environment chamber of the general type described by CHILDERS and BRODY (8) and AAMODT (1) was used.

A schematic diagram of the chamber is presented in figure 1. The chamber was located indoors and no provision made for other than artificial illumination. The light was supplied by 1000- or 1500-watt Mazda lamps which could be supplemented when desired by 500-watt mercury arc lamps. The major part of the heat from the lamps was dissipated by allowing water to flow over the glass plate which comprised the top of the chamber; the water depth could be varied from a few millimeters up to 3 centimeters.

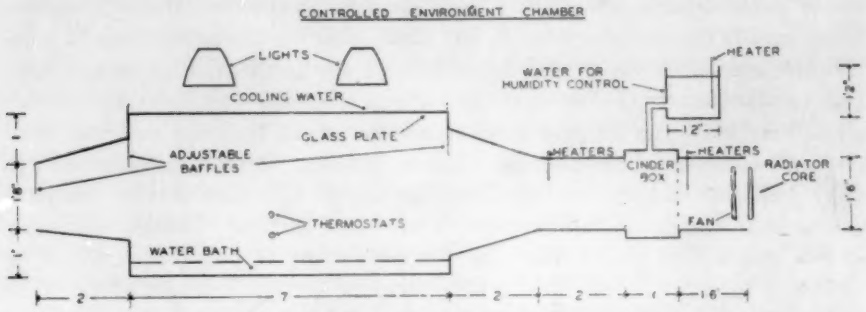


FIG. 1. Schematic diagram of controlled environment chamber.

The interior was painted white in order to utilize the light most efficiently. The light intensity could be varied by changing the size or number of lamps, the distance between the lamps and the top of the chamber, or the location of the plants within the chamber. In the present experiments intensities over 5000 foot-candles were not used and in most cases the plants were exposed to about 3000 foot-candles.

The temperature was maintained by means of the cold-water radiator and the heaters located as shown in the diagram. The heaters were operated by bimetallic thermoregulators located within the chamber, and the temperature controlled in this way. In most experiments different day and night temperatures were used so that it was convenient to avoid daily adjustment by having two thermoregulators, either of which could be thrown into the circuit by means of a toggle switch; one thermoregulator was set for the day temperature and the other for the night. For the present work the range 70° to 115° F. was of most interest and it was possible to maintain any given temperature within 2° F. in that range. If lower temperatures were to be employed it would be necessary to install a refrigeration unit. The lower part of the chamber was constructed so as to permit independent control

of root temperature by means of a water bath. In the present work, however, the soil and air temperatures were the same and this part of the apparatus was not used.

In many cases it was necessary to know the temperature in the immediate vicinity of the leaf. For measurements of this type, copper-constantan thermocouples of no. 32 wire were constructed (**12**) which could be placed on each plant in the desired position; a Leeds and Northrup Model K potentiometer was used and the readings could be easily made without disturbing the plants or the conditions within the chamber. A permanent time-temperature-humidity record was available from a centrally located self-recording hygrothermograph which was in operation at all times.

The air circulation was provided by a 12-inch fan located as shown in the diagram. The air movement within the chamber could be modified and regulated by means of the adjustable baffle plates; when increased air movement was desired, one to four 8-inch fans were used directly in the chamber. The humidity was controlled by allowing hot water to flow down through a compartment of cinders placed in the air stream. The heating of the water was thermostatically regulated, and the water level maintained by means of a ball float. The relative humidity could be varied from 30 per cent. to saturation, depending somewhat on the temperatures employed, and the external conditions.

Plant experiments

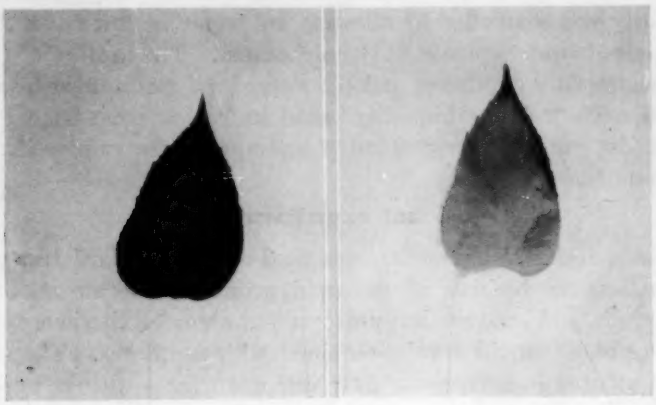
Sunflower, *Helianthus annuus*, was used as the standard test plant for these investigations because of its rapid growth, moderate sensitivity to spray materials, and its large and uniform leaf area. Experiments on other species were based on the results obtained with sunflower. The sunflower seeds were planted in greenhouse flats and when the seedlings were two or three inches high they were transplanted to 2-quart tin cans containing a good greenhouse compost.

Evaluation of injury

The injurious effects of spray emulsions applied to plants may be manifest in a variety of ways. In some instances the growing point or very sensitive young leaves are the only parts which may be injured, while in other cases the injury may be evident at the margin and tips of the older leaves and in still others the entire leaf blade may become brown or discolored. Oftentimes the injury is not accompanied by chlorophyll degradation as indicated by discoloration but is evidenced by physical changes in the leaf such as withering or loss of turgidity.

These various types and different degrees of injury are difficult to record and it became necessary to devise a system adequate to evaluate and describe the results. Photographs were found to aid materially in showing the extent of the injury, and in combination with a brief statement of observation were found to be more useful as a permanent record than detailed written descriptions alone. A photographic record was kept, therefore, in those

cases where definite and clear-cut injury was obtained with any particular emulsion under any given set of conditions. Ordinary black and white film was not satisfactory for this purpose since, with the type of injury usually obtained, lack of contrast made it difficult to distinguish the injured from the normal areas. Excellent contrast was obtained, however, by the use of infra-red film with the proper filter. Figure 2 shows a comparison of photographs of the same leaf obtained by employing (a) panchromatic, and (b) infra-red film. In this case the marginal injury was barely visible to the eye. Since chlorophyll does not absorb near infra-red radiation to any great extent, the normal areas of the leaf will appear light in the photograph, while the necrotic areas, in which degradation products have been formed, and which consequently do absorb in the infra-red region of the spectrum, will appear dark. It should be noted that with panchromatic film the normal leaf may appear dark because of the manner of printing which does not rep-



a b

FIG. 2. Sunflower leaf with marginal injury photographed (a) on panchromatic film; and (b) on infra-red film.

resent injury and should not be confused with the injury shown by the dark areas on prints from infra-red film. Necrotic areas which can be scarcely observed visually are clearly differentiated in the photographic print when infra-red film is employed. BAWDEN (2) has shown that foliar necrosis caused by certain virus diseases can be clearly demonstrated by the use of infra-red film.

For the routine photographing a Zeiss (maximar-6 × 9 cm.) camera was used with Eastman infra-red film. Number 1 photofloods were employed for illumination and a Corning no. 245 filter used in front of the lens. Under our conditions the proper exposure was about 1/25 second at F 8 but the correct exposure for any given conditions would have to be determined by trial and error.

As a matter of uniformity, and to provide a basis for the standardization of injury results on sunflower, the uppermost four well-developed leaves

were used as an index of the entire plant for the photographs and injury ratings (see figs. 3 and 4). After completion of the test run, the plants were carefully examined and observations relative to the general condition recorded. When photographs were to be made, the representative leaves were removed from the plant and placed under a glass plate on blotting paper for a few minutes until adjustments of the camera and lights were made. This treatment tended to prevent excessive curling when the leaves were placed in position under the camera. Injury ratings were made on the basis of the extent of injured area on the four leaves. Each leaf was assigned a rating of 25 so that a leaf that was entirely injured would be given a value of 25 per cent. The total percentages for the four leaves represented the rating of the whole plant. For instance in figures 3 and 4 the following ratings would apply (leaf pointing upward designated as no. 1 and others numbered in order clockwise):

	FIGURE 3a	FIGURE 3b	FIGURE 4a	FIGURE 4b
Leaf 1	1	11	5	23
Leaf 2	1	15	0	21
Leaf 3	0	21	9	25
Leaf 4	3	8	6	18
	5	55	20	87

The plants would then be recorded as suffering 5, 55, 20, and 87 per cent. injury, respectively. It was found that observations of injury based on this system were in close agreement with those based on the entire plant.

WATER LOSS MEASUREMENTS

When water loss data were to be obtained, the moisture content of the soil was held between 40 and 45 per cent. which represented 67 to 75 per cent. of its moisture holding capacity. The literature regarding transpiration as affected by soil moisture content has been comprehensively reviewed by MILLER (**20**). He concludes from the experimental evidence available that "a plant will transpire the maximal amount of water when the soil has sufficient moisture for good tilth, but an increase above that amount has no effect." In preliminary experiments using soil varying from 30 per cent. saturation to saturation it was found that transpiration was markedly reduced at the low moisture levels and that satisfactory growth was obtained in soil at about 70 per cent. of its moisture holding capacity. For our studies, therefore, soil at approximately 70 per cent. saturation was used throughout.

The procedure for the measurement of water loss was as follows: Sunflower seedlings were transplanted to cans containing 1200 to 1500 grams of soil at approximately 70 per cent. of its moisture holding capacity. Evaporation from the soil was reduced by the use of oilcloth fitted around the stem of the plant and tied smoothly over the top of the can. During the test runs the cans were brought back to their original weights by the addition of water every 24 hours, and in this way the soil was maintained near

70 per cent. saturation. It is recognized, however, that the soil moisture particularly in the immediate vicinity of the roots, probably varied between rather wide limits in spite of efforts to maintain it by weighing and replacement of the water lost. It should be noted, however, that since transpiration is generally lowered as the soil moisture is decreased and since in any given run the control plants lose more water than the treated plants, any error due to this fact will cause a lowered calculated efficiency of the treatment in reducing water loss.

The determination of the efficiency of the various treatments in reducing water loss were made by weighing and calculation after the method described by DUGGAR and COOLEY (**14**). The amounts of transpiration of the control plants and the plants to be treated were obtained for a standardization

TABLE I

MEASUREMENT OF EFFICIENCY OF WAX EMULSION IN REDUCING WATER LOSS FROM SUNFLOWER

DATE	GROUP A WATER LOSS	GROUP B WATER LOSS	GROUP A / GROUP B	GROUP B CORRECTED WATER LOSS	PERCENTAGE EFFICIENCY
	gm.	*gm.*		*gm.*	
12/ 4/42 to 12/ 5/42	529	413	1.28	532	
12/ 6/42	355	281	1.26	362	
12/ 7/42	372	277	1.34	357	
			Av. 1.29		
GROUP A, TREATED WITH WAX EMULSION 12/7/42 AT 4:00 P.M.					
12/ 8/42— 9 A.M.	33	109	.....	141	77
12/ 8/42— 4 P.M.	82	133	.....	430	81
12/ 9/42—10 A.M.	53	136	.....	175	70
12/ 9/42— 4 P.M.	72	260	.....	335	79
12/10/42— 9 A.M.	49	119	.....	154	68
12/10/42— 4 P.M.	87	328	.....	423	79

period under the experimental conditions. After treatment of the test plants the rates of transpiration were again measured for given time periods under the same conditions; after these latter values had been corrected for any differences which existed between the test and control plants before treatment, the efficiency of the treatment could be calculated. The following typical experiment will illustrate the method. Eight sunflower plants were divided into group A and group B of 4 plants each. These were placed in the test chamber which was maintained at about 106° F. and 3000 foot-candles light intensity during the day, and about 75° F. and no illumination at night. The loss of water from each plant was measured for three days and the total daily loss in grams for each group is presented in table I. The ratio of the group A water loss to the group B water loss is calculated for each day and the average value used to correct the group B measurements and so make them equivalent to the group A readings. After the third day

the plants in group A were sprayed with a wax emulsion and water loss measurements made twice daily. The values obtained for group B, the control plants in this case, were corrected by the average factor as determined from the 3-day standardization run and the efficiency of the treatment in reducing the water loss was then calculated.

None of these plants showed symptoms of burning, and this is in line with other similar experiments which indicated that 70 to 80 per cent. reduction in transpiration did not of itself cause direct foliage injury. It is interesting to note that the efficiency in reduction of water loss was greater during the day periods than during the night.

Eight plants were ordinarily used in each experiment and they were selected for uniformity in size and vigor. In routine runs two plants were used as the controls and the test plants standardized against them. Six plants could therefore be sprayed with a given emulsion for an experiment, or two emulsions could be tested using three plants each. The length of time required for each experiment varied with the objective of the investigation. In some work where the injurious effects of constituents of certain spray emulsions were sought, a few minutes to six or eight hours sufficed to furnish the information. In other cases the plants were kept in the chamber for several days for observations of injury and water loss and in some of the later experiments observations have been made over periods of four to five weeks.

The transpiration of sunflower as affected by soil temperature has been studied by CLEMENTS and MARTIN (9). They concluded from their data that transpiration varied little with soil temperature between 55° and 100° F. but that it dropped rapidly below 55° F. and approached zero at 32° F. Their plants started to wilt at about 40° F. CAMERON (7), on the other hand, concluded that the rate of water loss from young Valencia orange trees decreased markedly with decreasing soil temperature from 90° to 45° F.

No wilting or other signs of distress or injury were observed in the control plants in air temperatures as high as 112° F. and hence no effort was made to control the temperature of the soil in which the plants were growing.

Results

The plants were grown in the greenhouse under normal conditions and in most cases were placed in the chamber immediately after spray treatment. Untreated plants showed no harmful effects as a result of being removed from the greenhouse and placed immediately in the chamber at temperatures up to 110° F. and light intensities up to 5000 foot-candles. Plants sprayed with water showed no injury under these conditions so that it was not considered necessary to spray the control plants with water. This is in agreement with KRAMER (17) who reported that injury to leaves from exposure to sun while covered with water drops possibly never occurs.

The first requisite of a satisfactory spray material is that it will not cause injury to the plant. The study of the toxicity of the spray as a function

of chemical composition and environmental conditions is then of prime importance. Table II shows the effect of spraying sunflower plants with certain solutions and placing them under conditions of 110° F. and about 3000 foot-candles light intensity. These plants were very susceptible to injury under these conditions but in no case were the checks injured.

Investigation showed that the injury was due primarily to only one of the constituents of the commercial emulsion, ammonium linoleate. It may be noted from table II that while an ammonium linoleate solution at a concentration of 0.5 per cent. apparently caused no injury symptoms, the plants were definitely injured where the concentration was raised to 1.5 per cent. This substance, however, appeared to be more toxic when used in the emulsion, probably because the wax emulsions were more viscous than the ammonium linoleate solutions and built up a thicker film on the leaf. This

TABLE II

INJURIOUS EFFECTS OF CERTAIN MATERIALS ON SUNFLOWER

SPRAY MATERIAL	PERCENTAGE INJURY
Commercial wax emulsion—diluted 4×	90–100
Commercial wax emulsion—diluted 8×	90–100
Commercial wax emulsion—diluted 24×	10– 30
Ammonium linoleate—3.0%	90–100
Ammonium linoleate—1.5%	30– 50
Ammonium linoleate—0.5%	0
Sodium sulphate——3.0%	0
Ammonium sulphate—3.0%	50– 90
Ammonium nitrate—3.0%	50– 90
Ammonium acetate—3.0%	50– 90
Phosphate buffer—1.7%—pH 7.9	0
Phthalate buffer—1.5%—pH 4.0	0
Sodium salt of linseed acids—3.0%	30– 50
Ammonium hydroxide—10%	90–100
Ammonium hydroxide—0.8%	0

view is supported by the observation that when foliage is sprayed, the surface condition often causes the run-off to collect at the tips and edges of the leaves and it is here that injury is first apparent and most severe when injury occurs at all. These findings lead to the recommendation that whenever possible ammonia should not be used in sprays to be applied to tender green foliage. It may be observed from table II that both the ammonium and linoleate ions were causative agents in the injury and this effect was not merely due to pH conditions.

Extensive studies of this type have furnished much information which serves as a sound starting point in the development of emulsions possessing desirable characteristics. It may be readily ascertained whether or not a given material or combination of materials may be safely applied to green plant tissue. It is also possible to determine the maximum concentration of a toxic material which may be safely used in a given combination under given environmental conditions.

General spray practice has indicated that the environmental conditions at the time of application determine to a large extent the degree of injury which may be caused by a given treatment. Systematic investigation of some of these factors was necessary, particularly in connection with the type of spray emulsion used in this work. Experiments showed that the severity and rate of appearance of injury symptoms were markedly affected by variations in the procedure of spray application. This may be illustrated by the following experiment.

A series of sunflower plants was divided into 4 groups, sprayed with a commercial wax emulsion, and treated as described:

Group 1. Sprayed and immediately placed in the controlled environment chamber at 110° F. and 3000 foot-candles light intensity.

Group 2. Sprayed and allowed to stand at room temperature until the film was completely dried (4 hours at about 80° F.) before being placed in the chamber at 110° F. and 3000 foot-candles.

Group 3. Sprayed and then maintained at 70° to 75° F. and 3000 foot-candles for 24 hours before being placed in the chamber at 110° F. and 3000 foot-candles.

Group 4. These plants were not sprayed and were used as the checks in the chamber at 110° F. and 3000 foot-candles.

The plants in group 1 were 80 to 100 per cent. injured after 3 hours at 110° F. Those in group 2 showed very slight injury after 3 hours at the high temperature but were 60 to 70 per cent. injured after 18 hours. The plants in group 3 were not injured after 3 hours at 110° F. and showed only very slight injury after 18 hours at this temperature. The check plants, group 4, showed no injury.

In experiments designed to evaluate the toxicity of emulsions it is desirable that the most severe conditions be employed since this essentially increases the sensitivity of the method. For this reason it has been our practice to place the plants in the controlled environment chamber immediately after application of the spray; this treatment is comparable to that given group 1 of the preceding experiment. For practical field work consideration should be given to the advantages of having the treated plants maintained at a lower temperature for the period immediately following the spray application, as in group 3.

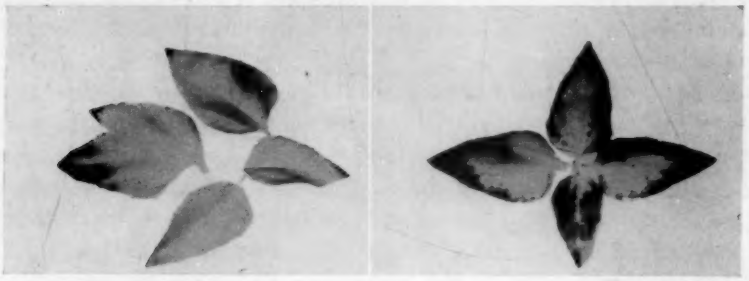
Figures 3 and 4 show the effect of temperature on the appearance of injury symptoms on sunflower. The group of plants represented by figure 3a was sprayed with oil emulsion H–1–54–5 and maintained at 102° F. The group illustrated by figure 3b was sprayed with the same emulsion and was treated identically except that the temperature was maintained at 109° F. This experiment was repeated using a slightly different emulsion, H–1–54–3, and the results are illustrated in figure 4. These plants were kept in darkness after having been sprayed, and the observations were made after 18

hours. The plants exposed to 109° F. were injured to a much greater extent than those at the lower temperature. This sensitivity to temperature has been substantiated by repeated experiments and is considered to account for some of the discordant field test observations of past years. Similar sensitivity to light intensity and humidity was not found. This work also indicated that emulsion H–1–54–3 was more injurious to the plants than emulsion H–1–54–5.

A series of emulsions of different compositions was prepared and the efficiencies of these emulsions in reducing water loss from sunflower were obtained by the procedures described with the following results:

Emulsion number	Percentage efficiency after two days	Wetting ability on leaves
	%	
P–6–46–1	22	Poor
P–6–44–1	13	Poor
P–6–48–1	33	Poor
P–6–34–1	53	Poor
P–6–40–1	49	Poor
P–6–50–1	49	Poor
P–6–42–1	51	Good
P–6–36–1	43	Good
P–6–38–1	43	Good

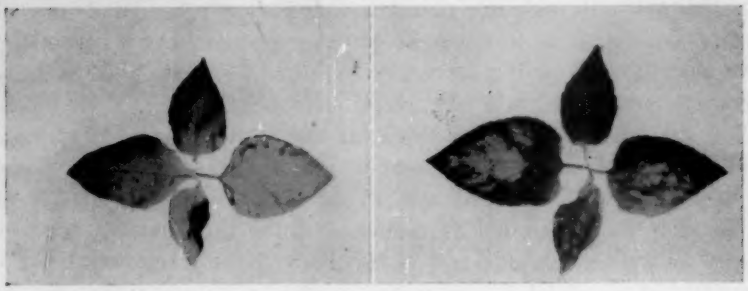
With the exception of the first two materials listed, there was no important difference in the efficiencies of the emulsions. The criteria of wetting ability were based on observation of coverage as the material was sprayed on the leaves. In no case were there symptoms of foliage injury.



a b

Fig. 3. Leaves from sunflower plants sprayed with oil emulsion H–1–54–5. (a) maintained at 102° F., (b) maintained at 109° F.

Table III contains data from a typical series of experiments designed to establish the performance of a given emulsion on sunflower and to determine the effect of a modification in the formula. Each injury and efficiency rating was obtained from a different experimental run and the values represent the averages of the measurements on the individual plants. The first group of eleven emulsions included different preparations of the same basic formula while the group of four emulsions represented a modi-



a b

FIG. 4. Leaves from sunflower plants sprayed with oil emulsion H–1–54–3. (a) maintained at 102° F., (b) maintained at 109° F.

fication of the formula. The controlled environment chamber was maintained at about 110° F. and 3000 foot-candles light intensity and the plants were placed in the chamber immediately after treatment. The data illustrate the usual degree of reproducibility obtained in this type of experiment and indicate the increased efficiency of the emulsion due to the formula modification.

It must be emphasized that water loss data have no meaning where the plants have been injured to any appreciable extent. For instance, in one experiment sulphonated castor oil was used in the oil emulsion and the plants, according to the usual measurements, showed over 90 per cent. injury and about 95 per cent. efficiency in the reduction of water loss; obviously, the foliage injury was directly responsible for the major part of the water loss reduction and no rating could be given the spray material in this regard.

TABLE III

INJURY AND EFFICIENCY RATINGS OF EMULSIONS ON SUNFLOWER

EMULSION NUMBER	NUMBER OF PLANTS TREATED	PERCENTAGE INJURY	PERCENTAGE EFFICIENCY
		%	%
H–1–58–1	2	1	44
H–1–60–1	2	10	38
H–1–85–3	2	1	33
H–1–87–5	2	8	32
H–1–91–1	6	0	63
H–1–99–1	3	0	43
H–1–75–1	5	1	41
H–1–79–1	4	13	22
H–1–83–1	2	2	36
H–1–101–1	2	20	57
H–1–105–1	3	1	47
		Av. 5	Av. 41
H–1–89–1	6	0	69
H–1–93–1	7	10	89
H–1–77–1	6	10	82
H–1–81–1	3	1	89
		Av. 5	Av. 82

As already indicated, the plant experiments are designed to evaluate emulsions developed as a result of physico-chemical investigations. The latter are concerned primarily with: (a) the preparation of satisfactory stable emulsions from materials which have been found non-injurious to the plant and which are readily available on the market and (b) studies on the relations between chemical composition, the most effective concentrations to be employed, the thickness of the film, the wetting ability of the emulsion, the adherence and permanence of the film, its resistance to removal by water, and the efficiency of the film retarding the passage of water vapor. Details of this work are to be reported elsewhere. Methods for the determination of the permeability of these spray coatings to water vapor have been reported by COMAR and MILLER (10) and in general the results obtained by the laboratory method parallel the efficiency ratings as determined by the use of plants.

These investigations have led to the development of formulas which are effective in reducing water loss from plants for short periods of time (2 to 3 days) without apparent injury. In experiments with certain plants these effects have been observed to last for as long as a month from a single treatment, at the end of which time the treated plants appeared to be in as good condition as the controls. It is realized, however, that before any statements can be made as to the benefits of such treatment on non-dormant plants it must be demonstrated that the use of these emulsions does not interefere with photosynthesis, respiration, or plant functions other than transpiration. Extensive field tests which are expected to show any effects of this treatment on yield are now under way and investigations of the physiological functions as affected by the use of wax and oil emulsions are planned as a necessary phase in the continuation of this program.

Summary

A controlled environment chamber is described and methods presented for the evaluation of the effects of wax and oil emulsions on plants as a function of environmental conditions and emulsion formula. Infra-red photography was found useful in the measurement and illustration of foliage injury when such injury involved chlorophyll destruction.

Methods for the determination of efficiency and injury ratings using sunflower as a standard test plant are described. Data are presented which illustrate how information can be obtained and used to determine and eliminate many of the problems, and how it is possible to design experiments which lead to the systematic formulation of satisfactory emulsions.

The authors wish to express appreciation to H. G. PETERING and O. M. NEAL who designed and built the controlled-environment chamber used in this work.

MICHIGAN AGRICULTURAL EXPERIMENT STATION
EAST LANSING, MICHIGAN

LITERATURE CITED

1. AAMODT, O. S. A machine for testing the resistance of plants to injury by atmospheric drought. Canadian Jour. Res. **12**: 788–795. 1935.
2. BAWDEN, F. C. Infra-red photography and plant virus diseases. Nature **132**: 168. 1933.
3. BENNETT, H. New commercial emulsifying agents. Ind. & Eng. Chem. **22**: 1255–1256. 1930.
4. BRIGGS, LYMAN J., and SHANTZ, H. L. The water requirements of plants. I.—Investigations in the Great Plains in 1910 and 1911. U. S. Dept. Agr. Bur. Plant Indust. Bull. 284. 1913.
5. ————, and ————. The water requirement of plants. II.—A review of the literature. U. S. Dept. Agr. Bur. Plant Indust. Bull. 285. 1913.
6. ————, and ————. Relative water requirement of plants. Jour. Agr. Res. **3**: 1–64. 1914.
7. CAMERON, S. H. The influence of soil temperature on the rate of transpiration of young orange trees. Proc. Amer. Soc. Hort. Sci. **38**: 75–79. 1941.
8. CHILDERS, N. F., and BRODY, H. W. An environment-control chamber for study of photosynthesis, respiration, and transpiration of horticultural plants. Proc. Amer. Soc. Hort. Sci. **37**: 384–390. 1939.
9. CLEMENTS, F. E., and MARTIN, E. V. Effect of soil temperature on transpiration in *Helianthus annuus*. Plant Physiol. **9**: 619–630. 1934.
10. COMAR, C. L., and MILLER, E. J. A method for the determination of permeability of agricultural spray coatings to water vapor. (In press.)
11. CURTIS, OTIS F. What is the significance of transpiration? Science (n.s.) **63**: 267–271. 1926.
12. ————. Leaf temperatures and the cooling of leaves by radiation. Plant Physiol. **11**: 343–364. 1936.
13. DIXON, HENRY H. Transpiration and the ascent of sap in plants. Macmillan and Co. Ltd. London. 216 pp. 1914.
14. DUGGAR, B. M., and COOLEY, J. S. The effect of surface films and dusts on the rate of transpiration. Ann. Missouri Bot. Gard. **1**: 1–22. 1914.
15. EMERSON, J. L., and HILDRETH, A. C. Preliminary report on reducing transpiration of transplanted evergreens. Science (n.s.) **77**: 433–434. 1933.
16. KIESSELBACH, T. A. Transpiration as a factor in crop production. Nebraska Agr. Exp. Sta. Res. Bull. 6. 1916.
17. KRAMER, PAUL J. The effect of drops of water on leaf temperatures. Amer. Jour. Bot. **26**: 12–14. 1939.
18. LIVINGSTON, BURTON E. Influences that affect transpiration from plant leaves. Sigma Xi Quar. **26**: 88–101. 1938.
19. MANEY, T. J. An apparatus for spraying plants with melted paraffin or other waxes. Proc. Amer. Soc. Hort. Sci. **28**: 496–497. 1931.

20. MILLER, EDWIN C. Plant physiology. McGraw-Hill Book Co. Inc., New York. pp. 407–522. 1938.
21. MILLER, E. J., NEILSON, J. A., and BANDEMER, SELMA L. Wax emulsions for spraying nursery stock and other plant materials. Michigan Agr. Exp. Sta. Spec. Bull. 282. 1937.
22. MORRIS, R. T. Nut growing. Macmillan Co. New York. 1921.
23. NEILSON, J. A. Paraffin wax—an aid to growth in transplanted trees and shrubs. Proc. North. Nut Grow. Ass'n. **19**: 44–51. 1928.
24. ————————. Reducing storage and transplanting losses in nursery stock. Florida Exch. and Hort. Trade World **78**: (no. 5) 27, 35. 1931.
25. TUKEY, H. B., and BRASE, KARL. The effect of paraffining, pruning and other storage treatments upon the growth of roses and cherry trees. Proc. Amer. Soc. Hort. Sci. **28**: 489–495. 1931.
26. WILSON, A. L. Triethanolamine emulsions. Ind. & Eng. Chem. **22**: 143–146. 1930.

EFFECT OF BORON, COPPER, MANGANESE, AND ZINC ON THE ENZYME ACTIVITY OF TOMATO AND ALFALFA PLANTS GROWN IN THE GREENHOUSE[1]

L. F. Bailey and J. S. McHargue

(WITH FIVE FIGURES)

In recent years much effort has been made to ascertain the necessity of certain so-called minor elements in the economy of plants and animals. It is only recently that attention has been given to physiological aspects of minor-element nutrition. The present investigation was begun to ascertain the effect of copper, boron, manganese, and zinc on enzyme activity in the plant. Catalase, peroxidase, oxidase, and invertase were included in the study.

The literature of the effect of minor elements on enzymes in plants is meager. Alexander (**1**) found that the catalase activity in squash plants was increased by boron deficiency. Haas and Klotz (**8**) observed a slight increase in diastatic digestion in citrus plants in response to boron deficiency. It has been suggested that manganese may have certain stimulating "catalytic" functions, such as inactivation of oxidizing enzymes (**11**, p. 112).

Wynd (**19**) studied the effect of small amounts of iodine on enzymatic activity in tomatoes. Low concentrations of iodine (1 and 5 p.p.m.) decreased invertase, peroxidase, and catalase activity. Higher concentrations (10 and 20 p.p.m.) produced marked increases in these enzymes. Oxidase activity was depressed with all treatments. Peptase gave no response to any of the iodine additions.

Methods

Marglobe tomatoes were used for the study of the effect of copper on enzyme activity. The plants were grown in the four-salt nutrient solution of Arnon (**2**) with boron, manganese, zinc, and iron added in appropriate quantities. The details of this culture solution have been described elsewhere (**3**). Thorough purification of the stock solutions was attained by the technique of Steinberg (**17**), and Stout and Arnon (**18**). This procedure involves the precipitation of heavy metals as phosphates under alkaline conditions. It was repeated to insure more complete removal of copper.

The tomato plants were grown in porcelain dye pots having a capacity of 1300 ml. One plant was grown in each pot and three replications were used for each treatment. The treatments consisted of no added copper, and additions of 0.01, 0.05, and 0.1 p.p.m. of copper as $CuSO_4$. The plants were grown to maturity before enzyme analyses were begun. For some time the

[1] Contribution from the Chemistry Department of the Kentucky Agricultural Experiment Station, Lexington, Kentucky. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

plants to which no copper was added showed severe copper deficiency (**3**). All copper treatments resulted in excellent growth, although this growth was somewhat restricted in the series receiving 0.01 p.p.m. copper.

Enzyme analyses were made in sets of four with each treatment represented and comparative results for the four treatments were thus obtained. Leaves and fruits were analyzed for enzyme activity. In each case it was found that comparisons of different treatments were possible only when the ages of the individual samples were the same. For the fruits, the age was assumed to be the same when they showed a similar stage of ripeness. The most satisfactory comparisons were obtained with mature leaves taken from nodes of similar age on the different plants. Since the copper-deficient plants grew very slowly, the leaves at the growing tips of these plants were much older than those of the more vigorous plants; consequently, it was not possible to select young leaves of comparable age from the different treatments. Only a few small fruits were produced on the copper-starved plants which limited the number of tests made on fruits of this treatment.

The tomato plants were grown during the summer of 1942. The following winter, alfalfa was grown and used in a similar study of the effect of boron, manganese, and zinc on enzyme activity. These plants were grown in two-gallon earthenware pots filled with acid-washed sand. The nutrient solution was supplied to the plants by the continuous-flow method (**16**). This solution was similar to the one used for tomatoes. Zinc was added as $ZnSO_4$ with one set having no zinc added, and others receiving 0.5, 1, and 2 p.p.m. Boron treatments included additions of 0.1, 0.25, 0.5, and 1 p.p.m. with the boron supplied as H_3BO_3. One set of plants had no manganese added while two others received 1 or 2 p.p.m. of manganese as $MnSO_4$. Thirty pots of alfalfa were used in this study, each pot containing 10 plants. Three replications were used for each treatment.

Enzyme analyses were made simultaneously for all treatments of a particular element. In this manner comparable results were obtained for the different treatments of this element. Only the first crop of alfalfa was used, a representative sample of this being taken for each enzyme test.

In the preparation of the tomato plants for catalase, peroxidase, and oxidase studies the plant material was ground in a porcelain mortar with sand. This material was then filtered through linen and the juice diluted to a suitable volume with water. In the later studies with alfalfa, maceration was accomplished with a Waring Blendor with water added at the rate of 15 ml. to each gram of leaf. The latter procedure was better in that it permitted larger amounts of material to be handled more quickly.

Determination of catalase

Catalase activity was determined gasometrically with an apparatus similar to that illustrated by Davis (**5**). The plant material was thoroughly coated with a $CaCO_3$ paste before maceration. A powdered c.p. grade of $CaCO_3$ proved satisfactory for this purpose. For leaves, one-half gram of

$CaCO_3$ was used for each gram of leaf; for tomato fruits, the ratio of carbonate to fruit was 1 to 5. After grinding, the plant material was filtered through linen with suction. The juice from tomato leaves (ground in a mortar) was diluted and mixed with 20 volumes of water. A portion of this water was used in the $CaCO_3$ paste used to coat the leaves. The alfalfa material, to which some water had been added prior to maceration in the blendor, was further diluted with a volume of water equivalent to the filtered extract. Tomato-fruit extracts had much less catalase activity and no dilutions of these were made.

One ml. of the plant juice was added to the reaction bottle and 5 ml. of 3-volume, neutral H_2O_2 were placed in the separatory funnel above the reaction bottle. Both the reaction bottle and the H_2O_2 were kept in a constant-temperature water bath at (25° C.) prior to their use. The reaction unit was then attached to a mechanical shaker immersed in the water bath. When the burette was adjusted, the H_2O_2 was admitted to the reaction chamber and the oxygen evolved measured after three minutes. The shaker accommodated two reaction units which permitted duplicate analyses to be made simultaneously.

It was necessary to standardize the procedure for all tests by using a constant time interval between the beginning of grinding and the measurement of the oxygen evolved. This standardization was necessary because of the continuous decrease in catalase activity after extraction. HEINICKE (**9**) found that catalase of apple leaves increased after extraction for about an hour, and then remained stable for as long as several days. In numerous tests made on tomatoes and alfalfa in this study, catalase activity always decreased after grinding, regardless of the amount of $CaCO_3$ used to neutralize acidity. The use of a standard time interval of 12 minutes between grinding and analysis permitted comparable results to be obtained from the different tests.

Several methods of neutralizing the acids released in maceration were tried in an effort to prevent the loss of catalase at the time of maceration and thereafter. Calcium carbonate in water gave unsatisfactory results, as did a phosphate buffer (pH 7.0). Dry Na_2CO_3 was more effective but was not as satisfactory as dry $CaCO_3$. HEINICKE (**9**) used equal weights of $CaCO_3$ and leaf material. In this study one-half this quantity of carbonate was used for leaves although the larger quantity recommended by HEINICKE was equally satisfactory.

The use of the Waring Blendor for maceration suggested that the metal blades might inhibit catalase activity; however, extracts prepared with the blendor and with a porcelain mortar gave similar results in several tests. The use of scissors or metal knives to chop the material produced a marked inhibition of this enzyme which did not occur when a plastic knife was used.

DETERMINATION OF PEROXIDASE

The preparation of the plant material for peroxidase tests was similar to the catalase procedure except that no $CaCO_3$ was used. Maceration in the

Waring Blendor was more satisfactory than grinding in a mortar. The tomato leaf extract was diluted with 200 volumes of water, and the less active fruit juice was diluted 1 to 2. The alfalfa received 15 ml. of water per gram of material before blending, and the filtered extract was further diluted to 50 volumes. One ml. of each dilution was used for peroxidase analyses, and each sample was tested in duplicate.

Peroxidase was determined by the method of GUTHRIE (**7**) in which indophenol formation from the oxidation of alpha-naphthol and para-phenylenediamine hydrochloride is utilized. At neutrality autoxidation of this mixture is very rapid. At pH 4.5 it is sufficiently slow to permit its use as a substrate for peroxidase measurement. The use of a citrate buffer at pH 4.5 does not prevent peroxidase activity but catalase and oxidase interference are eliminated. The substrate used in this study consisted of 200 ml. of buffer, 200 ml. of water, 1 gram of para-phenylenediamine hydrochloride, and 20 ml. of four per cent. alpha-naphthol in 50 per cent. alcohol. After filtering, 15 ml. of this substrate was immediately placed in a 50-ml. centrifuge tube containing one ml. of plant extract. Three ml. of N/20 H_2O_2 were then added. The reaction was stopped after 15 minutes with three ml. of 0.1 per cent. KCN. The indophenol formed was extracted with 15 ml. of toluene with shaking, and the two liquid layers were separated by centrifuging. The toluene layer was then poured off and determined in a photoelectric colorimeter.

DETERMINATION OF OXIDASE

Considerable confusion exists in the use of the terms oxidase and oxygenase. A common conception is that oxidase is a system composed of two enzymes, oxygenase and peroxidase. Oxygenase is supposed to produce organic peroxides which are then acted upon by peroxidase. Recent evidence (**4**) indicates that peroxidase acts only on H_2O_2, and that secondary oxidations by the quinones formed from oxygenase activity do not require the presence of peroxidase or any other enzyme. Recent practice favors the use of the term oxidase for the enzymatic quinone formation.

In the present study oxidase refers to those oxidizing enzymes which form quinones that oxidize pyrogallol or para-phenylenediamine without the addition of H_2O_2. This probably limits the study to cytochrome oxidase and polyphenol (catechol) oxidase. The former acts only on reduced cytochrome (**12**) but this in turn will oxidize such substances as pyrogallol, para-phenylenediamine, catechol, ascorbic acid, hydroquinone, a mixture of para-phenylenediamine and alpha-naphthol ("Nadi reagent"), and others. Polyphenol oxidase (**13**) is specific for ortho-dihydroxy phenolic compounds, such as naturally-occurring tannins. These substances will then oxidize pyrogallol, catechol, para-phenylenediamine, the Nadi reagent, etc. These secondary oxidations serve for the colorimetric determination of oxidase activity. Plant extracts such as those used in the present studies contain the oxidases and their specific substrates, and these can be measured colorimetrically through secondary oxidation of the compounds mentioned above.

The use of the Nadi reagent for oxidase determinations is undesirable because of the rapid auto-oxidation of this reagent in neutral media. Acid conditions required to reduce this auto-oxidation also inhibit oxidase activity. Para-phenylenediamine undergoes auto-oxidation somewhat less rapidly. Catechol produces a distinct color change rather slowly. Pyrogallol appears to be the most satisfactory reagent for oxidase determinations (**12**). The product of this oxidation is the yellow-brown purpurogalline. Pyrogallol was used in this study of oxidase.

The plant materials were prepared exactly as described for peroxidase. The extracts of the tomato leaves, ground in a mortar, were diluted with

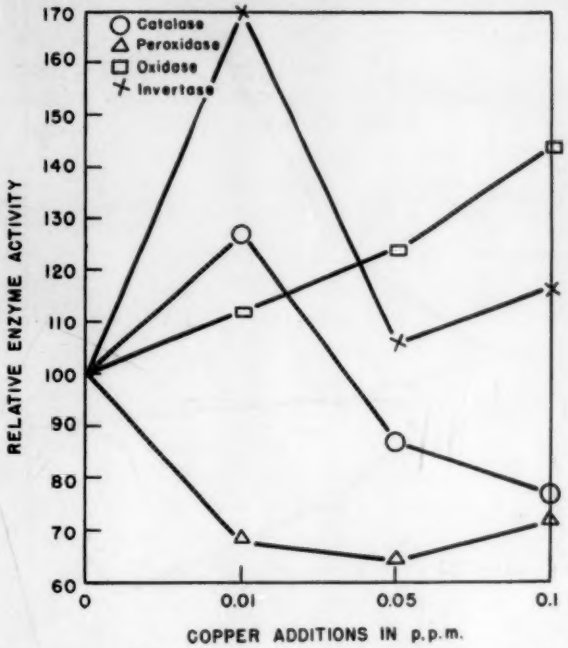


FIG. 1. Enzyme responses of tomato leaves to different amounts of copper added in nutrient solutions. The data are calculated as relative enzyme activities, the standard of comparison being 100 for the copper-starved plants.

50 volumes of water. The alfalfa material, after blending, was diluted to 10 volumes. The analyses were made on one-ml. portions of these dilutions and were run in duplicate.

To the plant extract 10 ml. of phosphate buffer (pH 7.3) was added and one ml. of one per cent. pyrogallol. After 10 or 15 minutes the mixture was examined in the colorimeter. The purpurogalline may be extracted into ether for colorimetric reading.

WYND (**19, 20**) adapted the previously described peroxidase procedure (Nadi reagent) to the measurement of oxidase by omitting the addition of H_2O_2. This presupposes that oxidase activity is not inhibited at pH 4.5. Repeated trials on tomato and alfalfa extracts with WYND's method failed

to give the typical reddish-purple color in toluene. A yellow-brown color was produced which did show some variation with the different treatments of the experimental plants. It seems doubtful that enzymatic oxidations are measured by this procedure. In tests with pyrogallol slight oxidase activity occurred at pH 4.5 but it was much less than that at pH 7.3. WYND (**19**) states that tomato fruits contain no oxygenase (oxidase) although the source

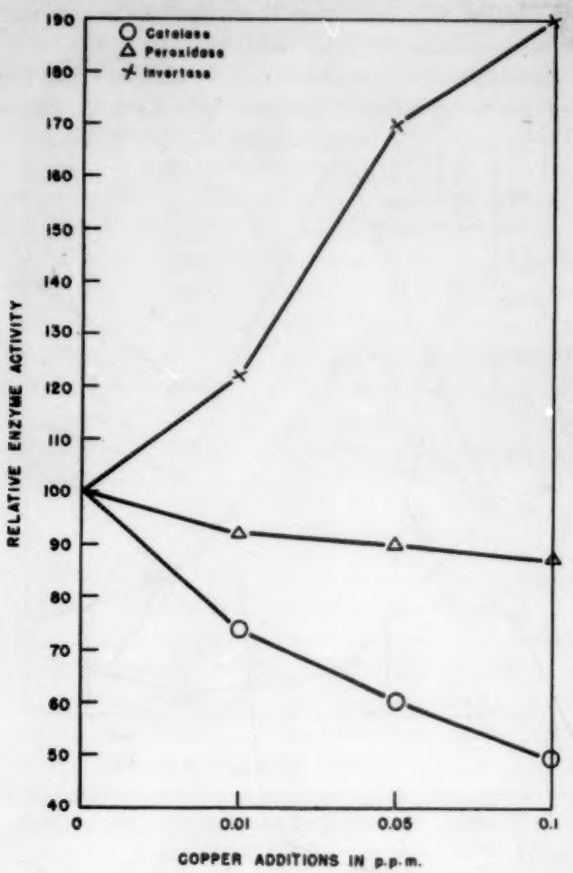


FIG. 2. Enzyme responses of tomato fruits to different amounts of copper added to nutrient solutions. The data are calculated as relative enzyme activities, the standard of comparison being 100 for copper-starved plants.

of this information is not given. Tests with tomato using pyrogallol indicate that considerable oxidase is present in the fruit.

DETERMINATION OF INVERTASE

The invertase procedure used was similar to the one described by WYND (**19**). The plant material was soaked overnight in absolute alcohol and washed in ether. It was dried before a fan for several hours and then in a vacuum desiccator over H_2SO_4 for 24 hours. After grinding in a mortar,

the material was brought to air-dryness before weighing the sample for enzyme analysis. Duplicate one-gram samples were placed in flasks containing 50 ml. of water and soaked overnight in a refrigerator. Fifteen grams of commercial sucrose was then added followed by another 50-ml. portion of water which was used to wash down the sides of the flask. This mixture was incubated at 38° C. for 24 hours. After incubation, the mixture was filtered and reducing sugars analyzed in two-ml. portions of the leaf materials and one-half-ml. portions of the fruit material.

Reducing sugars were determined by the Munson-Walker-Bertrand method described by LOOMIS and SHULL (**14**). Sintered porcelain crucibles were used for the filtration instead of asbestos filters.

TABLE I

RELATIVE ENZYME ACTIVITY IN TOMATOES AND ALFALFA IN RESPONSE TO DIFFERENT AMOUNTS OF COPPER, BORON, ZINC AND MANGANESE ADDED TO THE NUTRIENT SOLUTION

TREATMENT, P.P.M.	CATALASE	PEROXIDASE	OXIDASE	INVERTASE
Copper	Tomato leaves			
0.00	100	100	100	100
0.01	127	69	112	170
0.05	87	65	124	107
0.10	77	72	144	117
	Tomato fruits			
0.00	100	100	.....	100
0.01	74	92	.....	123
0.05	63	90	.....	170
0.10	49	89	.....	190
Boron	Alfalfa			
0.10	100	100	100	100
0.25	114	168	103	158
0.50	122	140	103	139
1.00	130	142	109	215
Zinc				
0.0	100	100	100	100
0.5	97	111	109	94
1.0	136	167	130	82
2.0	94	123	120	94
Manganese				
0.0	100	100	100	100
1.0	83	177	99	66
2.0	79	126	75	78

Results and discussion

EFFECT OF COPPER

The results for the effect of copper treatments on enzyme activity in the tomato are presented in table I, and in figures 1 and 2. Since comparative values, rather than absolute amounts, were the object of the study, all data are presented as relative enzyme activity, the standard of comparison being 100 for the copper-starved series of plants.

In the tomato leaves, maximum catalase and invertase activity occurred with 0.01 p.p.m. copper. With higher concentrations of copper these enzymes decreased in activity. Oxidase increased with each successive addition of copper. Only peroxidase failed to respond positively to any copper additions.

In the fruits, catalase and peroxidase activity diminished progressively with increasing copper while invertase gave the opposite response. Oxidase determinations were not made on tomato fruits.

The different enzyme responses of fruits and leaves to copper serves to emphasize the physiological individuality of vegetative and reproductive

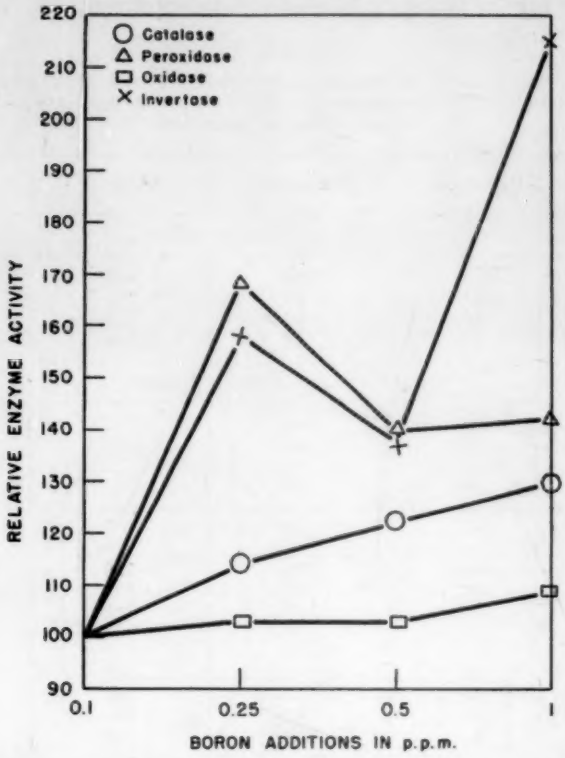


FIG. 3. Enzyme responses of alfalfa to different amounts of boron added in nutrient solutions. The data are calculated as relative enzyme activities, the standard of comparison being 100 for the 0.1 p.p.m. boron treatment.

portions of the plant. Peroxidase responded similarly in the two portions of the plant. Although there are points of similarity in the other curves, the different responses with fruits and leaves is striking.

Oxidase activity has especial interest here since it is now known that polyphenol oxidase is a copper-protein compound (**13**). The shape of this curve suggests that the amount of polyphenol oxidase present in the plant is a function of the available copper, and that the oxidase measured in these experiments probably was predominantly polyphenol oxidase.

ELVEHJEM (6) reports similar increases in oxidase activity in the liver of anemic rats in response to copper additions.

EFFECT ON BORON

The results for the relationship of boron to enzyme activity in alfalfa are given in table I and figure 3. The 0.1 p.p.m. treatment was used as a standard of comparison. All the enzymes studied gave maximum responses with 1 p.p.m. boron except peroxidase which reached maximum activity at 0.25 p.p.m. Plant growth with no boron added was insufficient for enzyme analyses and no treatments with boron additions greater than 1 p.p.m. were used.

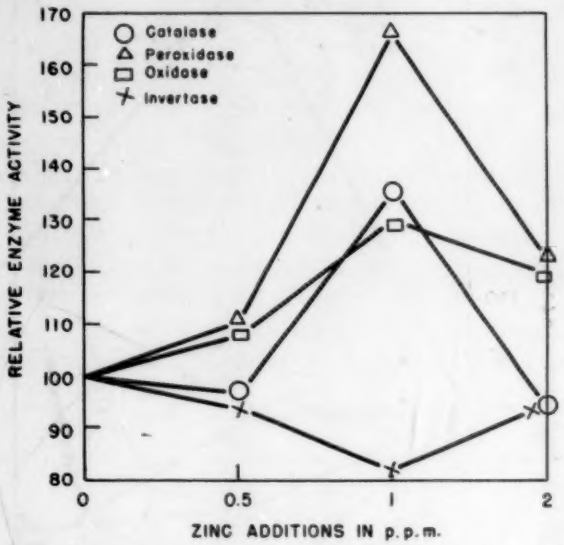


FIG. 4. Enzyme responses of alfalfa to different amounts of zinc added in nutrient solutions. The data are calculated as relative enzyme activities, the standard of comparison being 100 for the plants to which no zinc was added.

The invertase curve shows the most marked favorable response to boron with an increase of over 100 per cent. at the 1 p.p.m. boron level. No explanation is apparent for the peculiar drop in activity with 0.5 p.p.m. The boron stimulation of invertase cannot be attributed to an effect on the substrate since the substrate was added as commercial sucrose. These results for invertase suggest a possible relationship of boron to the carbohydrate mechanism of the plant through this enzyme.

EFFECT OF ZINC

Table I and figure 4 present the results for the effect of zinc on enzyme activity in alfalfa. Catalase, peroxidase, and oxidase gave pronounced optimal activities at 1 p.p.m. zinc. Invertase, on the other hand, shows minimum activity at this level. It is surprising that the critical level of enzyme activity should be at a zinc concentration as high as 1 p.p.m. The optimum level for growth is considered to be much lower than this.

Effect of manganese

The results for the effect of manganese on enzyme activity in alfalfa are given in table I and figure 5. The curves show a depressing effect of manganese at the concentration used with one exception. Peroxidase gave a marked favorable response to 1 p.p.m. manganese. The significance of this response is not clear. Peroxidase is an iron-porphyrin-protein enzyme. Since iron and manganese are closely related physiologically in the plant, it may be that manganese in some unknown manner affects the iron in the peroxidase molecule. The depressing effect of manganese on catalase,

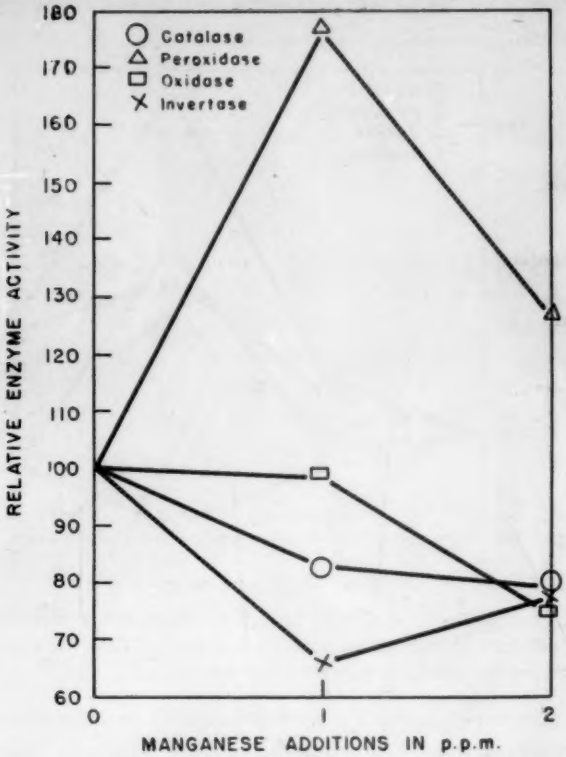


Fig. 5. Enzyme responses of alfalfa to different amounts of manganese added in nutrient solutions. The data are calculated as relative enzyme activities, the standard of comparison being 100 for the plants to which no manganese was added.

oxidase, and invertase cannot be explained unless it is possible that at some level between the limits of no added manganese and 1 p.p.m. there occurs a concentration which would stimulate enzyme activity.

General discussion

Examination of the curves of enzyme activity for any one of the minor elements shows considerable uniformity in the enzyme responses. Three enzymes show maximum activity with 1 p.p.m. zinc and three with 1 p.p.m. boron. For copper in tomato leaves two enzymes show their greatest re-

sponse at 0.01 p.p.m. Three enzymes are depressed by additions of 1 p.p.m. manganese. These results suggest that these enzyme responses are expressions of general metabolic conditions of the plant rather than direct influences of the particular minor elements on the enzymes. HEINICKE (**9, 10**) and NELLER (**15**) suggest that catalase activity measures the metabolic status of tissues and may serve as an indicator of the physiological responses of plants to their environment. It seems likely that any of the enzymes tested here may serve in a similar capacity; however, catalase seems to be most consistent in its agreement with other enzymes. WYND (**19**) found considerable uniformity in the responses of catalase, peroxidase, and invertase to iodine treatments. Oxidase and peptase were exceptional, but there is some question concerning the validity of the oxidase results of WYND.

There are certain exceptional enzyme responses for each of the minor elements used in this study. At least some of these exceptions may be explained as direct effects of one element on a particular enzyme. The response of oxidase to copper may be accounted for by the fact that polyphenol oxidase is a copper-protein enzyme. As discussed previously, manganese may have a direct effect on the iron prosthetic group of the peroxidase molecule, and boron may affect the carbohydrate mechanism of the plant through an invertase relationship. Similarly, the effects of zinc on invertase and of copper on peroxidase may be direct, although no explanation of these relationships is apparent at the present time.

Summary

1. Catalase, peroxidase, oxidase, and invertase determinations were made on leaves and fruits of tomatoes grown in nutrient solutions containing different amounts of copper. The same enzymes were determined for alfalfa plants grown with nutrient solutions containing different amounts of boron, manganese, and zinc.

2. The enzyme responses to copper treatments were not the same in leaves and fruits of tomato.

3. There was considerable uniformity in the enzyme responses for each minor element. This is interpreted as indicating that these enzyme activities are expressions of general metabolic conditions within the plants rather than direct influences of the minor elements.

4. Certain exceptional enzyme responses are considered as possible direct effects of an element on a particular enzyme. In this category is the continued increase of oxidase activity with increasing additions of copper.

KENTUCKY AGRICULTURAL EXPERIMENT STATION
LEXINGTON, KENTUCKY

LITERATURE CITED

1. ALEXANDER, T. R. Anatomical and physiological responses of squash to various levels of boron supply. Bot. Gaz. **103**: 475–491. 1942.

2. ARNON, D. I. Microelements in culture-solution experiments with higher plants. Amer. Jour. Bot. **25**: 322–325. 1938.
3. BAILEY, L. F., and MCHARGUE, J. S. Copper deficiency in tomatoes. Amer. Jour. Bot. **30**: 558–563. 1943.
4. BOSWELL, J. G., and WHITING, G. C. Oxidase systems in the tissues of higher plants. New Phytol. **39**: 241–265. 1940.
5. DAVIS, W. E. The use of catalase as a means of determining the viability of seeds. Boyce Thompson Inst. Professional Paper no. 2. 1926.
6. ELVEHJEM, C. A. The biological significance of copper and its relation to iron metabolism. Physiol. Rev. **15**: 471–507. 1935.
7. GUTHRIE, J. D. A method for the determination of peroxidase activity. Jour. Amer. Chem. Soc. **53**: 242–244. 1931.
8. HAAS, A. R. C., and KLOTZ, L. J. Further evidence on the necessity of boron for health in citrus. Bot. Gaz. **92**: 94–100. 1933.
9. HEINICKE, A. J. Factors influencing catalase activity in appleleaf tissue. Cornell Univ. Agr. Exp. Sta. Memoir 62. 1923.
10. ———. Seasonal variation in the nutrient condition of apple trees as indicated by catalase activity. Proc. Amer. Soc. Hort. Sci. **25**: 234–239. 1929.
11. Hunger signs in crops. The American Society of Agronomy and the National Fertilizer Association, Washington, D. C. 1941.
12. KEILIN, D., and HARTREE, E. F. Cytochrome oxidase. Proc. Roy. Soc. Lond. **B. 125**: 171–186. 1938.
13. ———, and MANN, T. Polyphenol oxidase. Purification, nature and properties. Proc. Roy. Soc. Lond. **B. 124**: 187–203. 1938.
14. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. McGraw-Hill, New York. 1937.
15. NELLER, J. T. Relation of catalase activity to physiological breakdown in Jonathan apples. Plant Physiol. **6**: 183–189. 1931.
16. SHIVE, J. W., and ROBBINS, W. R. Methods of growing plants in solution and sand cultures. New Jersey Agr. Exp. Sta. Bull. 636.
17. STEINBERG, R. A. Nutrient solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. Jour. Agr. Res. **51**: 413–424. 1935.
18. STOUT, P. R., and ARNON, D. I. Experimental methods for the study of the rôle of copper, manganese, and zinc in the nutrition of higher plants. Amer. Jour. Bot. **26**: 144–149. 1939.
19. WYND, F. L. The effects of increasing the iodine content of the tomato plant on respiration and enzymatic activity. Ann. Missouri Bot. Gard. **21**: 367–432. 1934.
20. ———. Certain enzymatic activities of normal and mosaic-infected tobacco plants. Jour. Gen. Physiol. **25**: 649–661. 1942.

RELATION OF LIGHT TO GROWTH OF PLANTS[1]

H. F. Thut and W. E. Loomis

(WITH EIGHT FIGURES)

Plants grown in varying intensities of light from full sun to darkness show characteristic and well-known differences in growth and development. Much of this effect is frequently summed up in the phrase, "light retards growth," which has become almost axiomatic in plant physiology and has been used to explain certain types of phototropism as well as the varying growth rates of etiolated and unetiolated plants, etc. Like many other physiological axioms, the inhibiting effect of light was popularized by Sachs (**20**) who performed some of the early experiments indicating that plants may make most of their growth in darkness. Sachs recognized the several secondary effects of light on growth, but thought it directly inhibiting through some action on the growing regions.

Numerous other workers have considered light inhibiting for growth. Prantl (**18**), Popp (**15**) and McCalla, Weir and Neatby (**13**) agree, at least in part, with Sachs. Mason (**12**) and Popp and Brown (**16**) have stressed the action of ultraviolet rays in checking growth. Mason found that date frond elongation stopped soon after sunrise at Indio, California, and was not resumed until near sunset. Growth was resumed within a few minutes if the plants were covered during the day, but could be stopped under the cover or at night by the radiation of a quartz mercury arc. Tropical workers, Brown and Trelease (**2**), Coster (**3**), Osmaston (**14**), Porterfield (**17**) and Trelease (**21**), are generally agreed that daytime checks of plant growth are caused by internal water deficits in insolated plants rather than by any direct action of light. It might be assumed that the lower percentage of ultraviolet in the sunlight of the humid tropics accounted for their observations if it were not that Lloyd (**9**) was unable to find any direct effect of sunlight upon the growth of *Eriogonum nudum* at the Desert Laboratory at Tucson.

Loomis (**11**), working with potted maize plants in the greenhouse, concluded that the elongation of this plant was controlled by the temperature and the internal water supply of the plant and was not directly affected by light. Temperature is normally positively correlated with radiation, but water supplies within the meristematic tissues tend to be negatively correlated. The greenhouse glass would reduce the ultraviolet of sunlight by screening out the shorter and more active wavelengths. Prescott (**19**), however, found that maize in the field in Egypt also made its greatest growth after sunrise in the morning and around sunset, with a midday drop that was accentuated by declining soil moisture percentages. There

[1] Paper no. J–1135 of the Journal series of the Iowa Agricultural Experiment Station. Project no. 678.

is thus a fairly general agreement among investigators that sunlight is not directly inhibitory for plant growth, although the verdict is not unanimous.

The present investigation was undertaken in the summer of 1939 to compare diurnal growth in the field with the earlier experiments on potted plants in the greenhouse, and to evaluate more accurately the various secondary effects of sunlight on growth. It was concerned with the effects of full sunlight on the growth of non-etiolated plants, so that the numerous complicated effects of light on plant form (**7, 22**) have been ignored.

Results

Experiments with corn

Plants of the open pollinated, Station strain of Iodent (*Zea mays*) were grown on the Botany plots of the Agricultural Experiment Station at Ames, Iowa, in a deep, black, fertile loam. The increases in height of the unfolding, central leaves of vigorous maize plants 10 to 15 dm. tall were recorded as growth. This measurement includes size increases due to cell division and elongation at the base of the leaf, in the leaf sheath, and in varying numbers of elongating internodes below the leaf. Preliminary measurements showed, however, that closely agreeing results could be obtained from three or four of the younger leaves on any one plant.

Measurements were made by fastening a ring with adhesive tape to the leaf to be measured. Auxanometer needles, balanced to a uniform pull of 15 gm., were then hooked into the rings, and elongation read with a 5 to 1 magnification on a meter scale. Auxanometers were detached when not making readings to avoid disturbance by wind. Humidity readings were made with a sling psychrometer, and light readings with a Weston Sunlightmeter. Hygrothermograph records were obtained from a recording instrument in the field to check and supplement the direct readings. In most of the experiments with both maize and other plants, readings were taken at 2-hour intervals during the experiment. Growth was calculated as millimeters per hour for an average of six or more plants and recorded as the growth rate at the middle of the period. Light, temperature, and humidity readings were plotted as taken.

The curves of figure 1 show the results of a four-day experiment, started June 23, with plentiful soil moisture and rising temperatures. The first two days were sunny, as shown by the light curve, but the third and fourth were partly cloudy, with showers. The growth rate, shown by the heavy black line, rose sharply after the cool morning of June 23, following the light and temperature curves until 10:00 A.M., then dipped slightly, and rose to a second peak at 10:00 P.M. At night the growth rate dropped with the temperature, rising to repeat the day pattern on June 24. The pattern for the showery days of June 25 and 26 is not so clear, but in general growth increased with the temperature in the morning, dropped with the humidity during afternoon, increased with the humidity in the evening and dropped with the temperature after 10:00 P.M. All the effects of day and night can thus be explained on the basis of two indirect effects of sunlight—temperature,

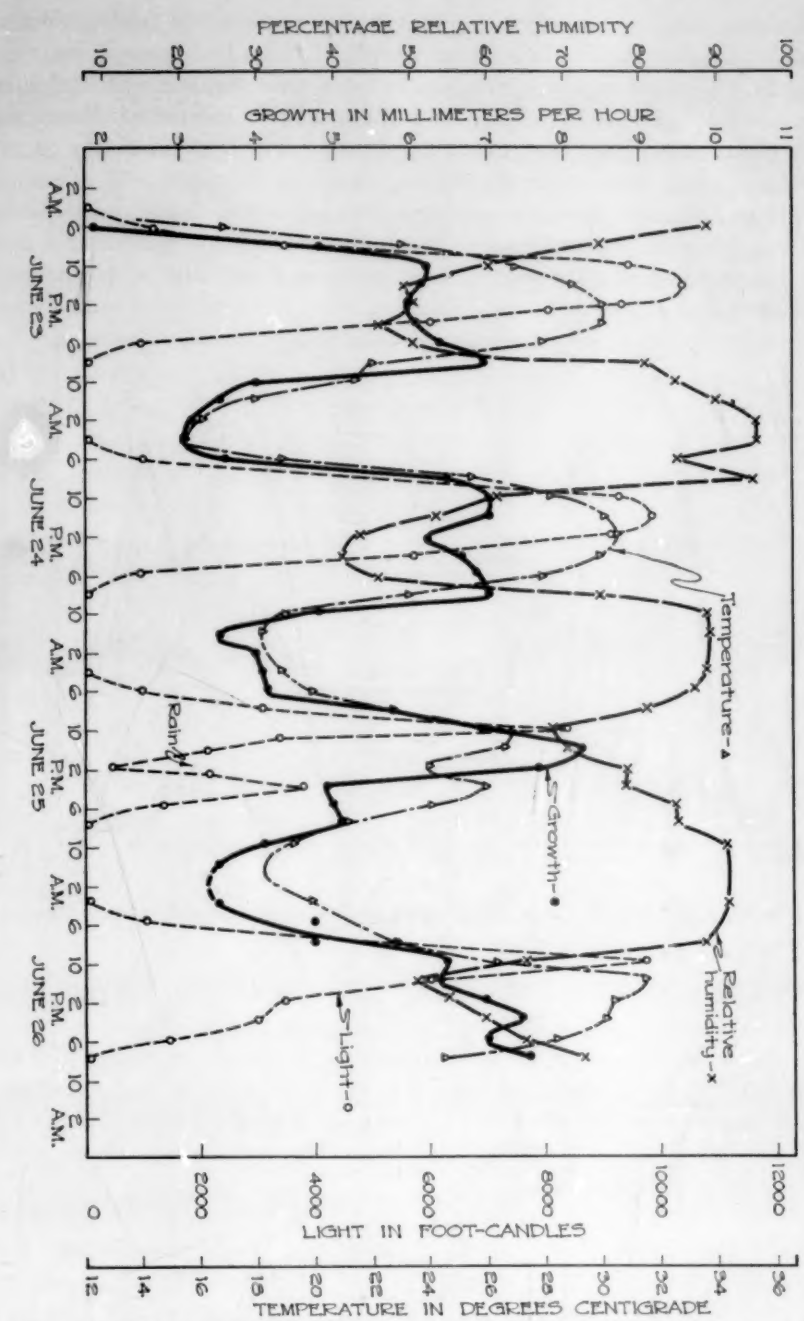


FIG. 1. **Light intensity, temperature and relative humidity in relation to the height growth of *Zea mays* under favorable moisture conditions.**

and water deficit—the latter as partially expressed by a falling relative humidity, and no direct effect on the growth of this plant is apparent. On June 24, for example, maximum growth rates were reached with full summer sunlight between 10:00 A.M. and 12:00 M. In individual plants this same phenomenon was encountered frequently. Sunlight intensities of 12,000 fc, obtained with banks of reflecting clouds to the north, did not check growth of *Zea mays* when temperatures were below 30° C. and soil moisture and relative humidity were high. Minimum soil moisture percentages during this experiment were above 22 per cent. in a soil with a wilting percentage of 9 to 10 per cent.

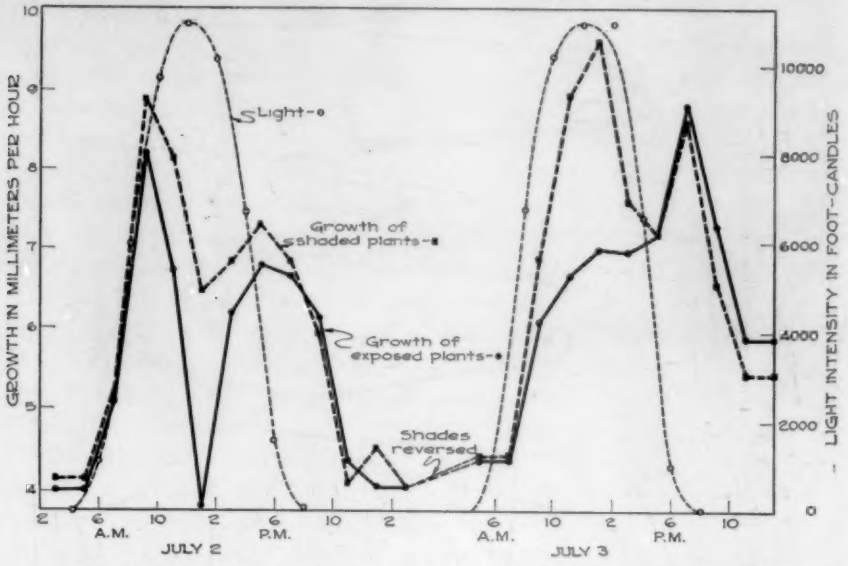


FIG. 2. Light intensity in relation to the growth of shaded and exposed plants of *Zea mays*.

These field results contrast with growth rates of well watered potted plants in the greenhouse (**11**) by showing much higher midday readings. Greenhouse humidities tended to be lower and temperatures higher with the same sunlight, but it seems probable that the restricted root systems of pot-grown plants were less effective in supplying moisture rapidly to the top than were the ramified, well developed root systems of the field plants growing in a deep, well drained soil.

EXPERIMENTS WITH SHADING.—Celotex boards were set to exclude direct sunlight from leaves of one set of six plants in a second experiment. The plants were otherwise fully exposed to north light and to air movement. The growth of these shaded plants, and of a second set exposed normally, is compared with the curves for sunlight and for temperature in figures 2 and 3. The two growth curves were essentially identical except for the greater growth of the shaded plants during the drier part of the day.

Considering the effect of light alone (fig. 2), the growth curves rose with light on the morning of July 2 and then dropped sharply at 1:00 P.M. Growth of the shaded plants rose somewhat higher and dropped to a minimum of 6.5 mm. per hour compared to 3.9 mm. for the exposed plants. Evening and night growth rates were obviously unrelated to light since growth increased rapidly between 1:00 and 5:00 P.M. and then dropped to a low value in the late evening. The shades were reversed at daylight on July 3, and the effects of the previous day's shading apparently reduced the morning growth rates of the newly exposed plants. The shaded plants made their maximum growth at 1:00 P.M., dropped to a daytime low at 5:00 P.M. and rose sharply with the unshaded plants to a second high at 7:00 P.M., just before sunset.

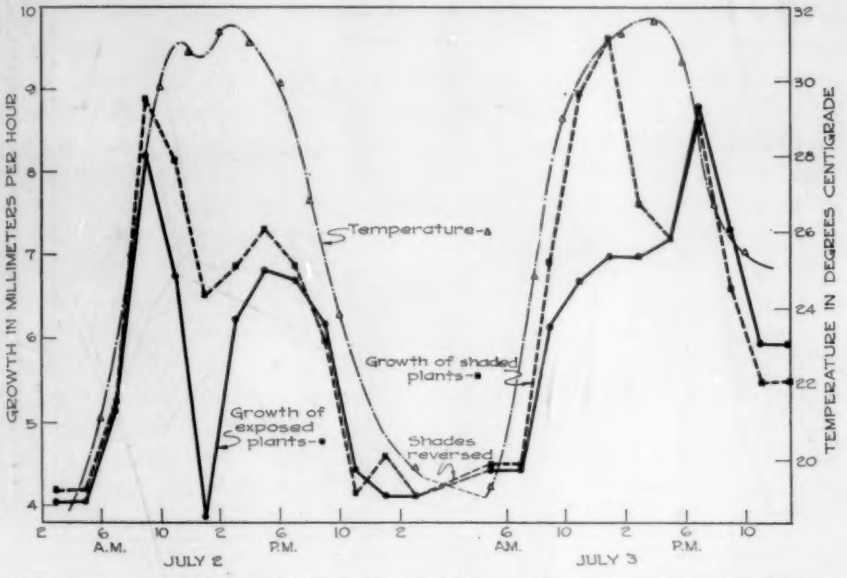


FIG. 3. Temperature in relation to the growth of exposed and shaded maize plants.

When these growth curves are compared with the air temperature curve (fig. 3), growth is seen to have followed temperature closely except during the driest part of the day, when the growth of the exposed plants was reduced more than that of the shaded ones.

Water deficits were measured directly in the shaded and unshaded plants by an eosin absorption method. Extra plants were shaded with celotex board for the measurements, and six control and six shaded plants were cut at two-hour intervals. The plants were immediately recut under water to eliminate plugging of the xylem by air, transferred to an eosin solution for 20 seconds, the top of the stalk cut away to stop intake of the dye, and the base split to measure the upward movement of the dye. With practice, dye absorption time could be controlled within one second and concordant results obtained. Maximum rates of dye movement shown in figure 4 are over 100

cm. per min., and rates of nearly 200 cm. have been observed. These figures do not represent normal rates of water movement in the plants, which usually are estimated in centimeters per hour, but indicate a sudden movement of water through the xylem and into the parenchyma of the plant when tension is released at the bottom of the columns. With dewfall, night-time dye absorption drops to zero, and midday readings of 6 cm. per min. were obtained by holding cut plants in water and shade for 30 min. before transferring to the dye.

In figure 4 the eosin injection figures are inverted, as records of water deficits within the plants, and plotted above the growth curves. In general they explain the failures of the growth curves to follow the temperature (fig. 3). The greater eosin uptake and lower midday growth rate of the

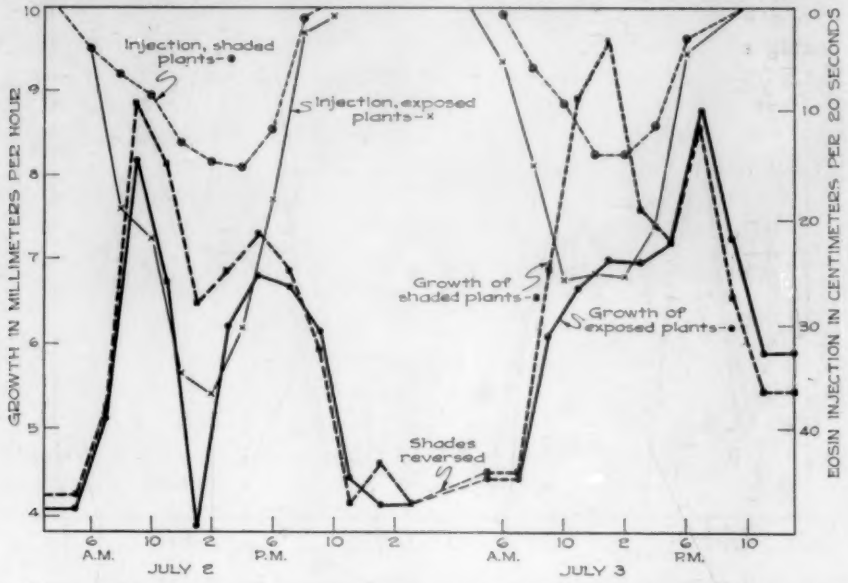


FIG. 4. Eosin injection, as a record of internal water deficit, and growth of maize.

unshaded plants on July 2 is explained as due to the drying effects of a brisk southwest wind. The later and higher growth peak of the shaded plants on the second day is not reflected in the injection curves and may have been due to chance, or to failure of the cut plants to show accurately the conditions within the nearby growth block.

This experiment also indicates that temperature is the main controlling factor for elongation in maize, with water deficit the opposed factor. When moisture did not become limiting, the growth rates shown in figures 1 and 3 doubled for temperature increases of 10° C. between the limits of 15° and 30°. Earlier and unpublished work (**8, 11**) indicates that 10° C. is the minimum for growth of maize and that 30° is near the optimum.

In a second shading experiment six plants were covered with heavy paper until maximum light intensities were 1 to 3 fc. The first day after shading

the covered plants grew four times as fast as the controls between 12:00 M. and 2:00 P.M., but the daily total for the two groups was the same, the control plants having made a much greater evening growth. By the third day of continuous darkness, growth of the covered plants began to slow from starvation, shown in low sugar percentages, dropping to less than 1 mm. per hour on the fifth day, while growth rates for the controls varied between 2 and 9 mm.

EFFECTS OF DRIER WEATHER.—If the midday dip in growth rates is the effect of water deficits, and the night time drop is due to low temperatures, hotter, drier weather should increase the night and decrease the daytime growth rates until the classical picture of plants growing at night is obtained. An approach to such a record was obtained on July 12–13 (fig. 5) when the maximum temperature was 37° C., the minimum 24.5°, and the soil moisture immediately under the hills was 3 to 4 per cent. above the wilting point. Moisture midway between hills was 4 per cent. higher and near the field capacity. DAVIS (**5, 6**) has shown, however, that soil moisture near the plant is removed preferentially, even when as here, fully developed root systems are present in moist soil farther away, and that soil moisture percentages well above the permanent wilting point may sharply check the growth of rapidly transpiring plants.

On July 12 the growth of maize, as shown by the heavy line in figure 5, dropped in the morning until the rate at 2:00 P.M. was about one-third of that at 6:00 A.M. The relative humidity was no lower than on June 24 (fig. 1) when the 2:00 P.M. growth rate was nearly five times as great. The eosin injections, however, showed a considerably greater internal water deficit in the slowly growing plants, with a figure of 44 cm. in 20 seconds at 2:00 P.M. of July 12. Temperatures, too, were probably excessive, although it is difficult to distinguish between the effects of high temperatures and high internal water deficits under field conditions. By 8:00 P.M. the temperature had dropped to 30° C., the eosin injection to 5 cm., and the plants were growing at a rate of 8 mm. an hour. By midnight the rate was down to 5.5 mm., dropping with the temperature and possibly with the completed elongation of an accumulation of freshly divided cells. The daytime pattern of July 13 was essentially the same as that of July 12 except that partial cloudiness maintained the 2:00 P.M. minimum at a higher rate. The night temperature, however, was 8° or 9° C. below that of the previous night, and the night growth rate was down to less than half. Also the growth rate rose with the temperature on the morning of July 14. The actual rates, however, were nearly identical with those obtained at the same time and with the same temperature on the previous day. July 14 temperatures were not excessive, and the poor daytime growth is explained again by the eosin injection figures which reached the high value of 35 cm. by 10:00 A.M. These plants showed no tendency to wilt at any time during the experiment; the soil moisture did not reach the wilting percentage under the hills, and the second foot of soil between the hills, well filled with roots, was still near field capacity at the end of the experiment.

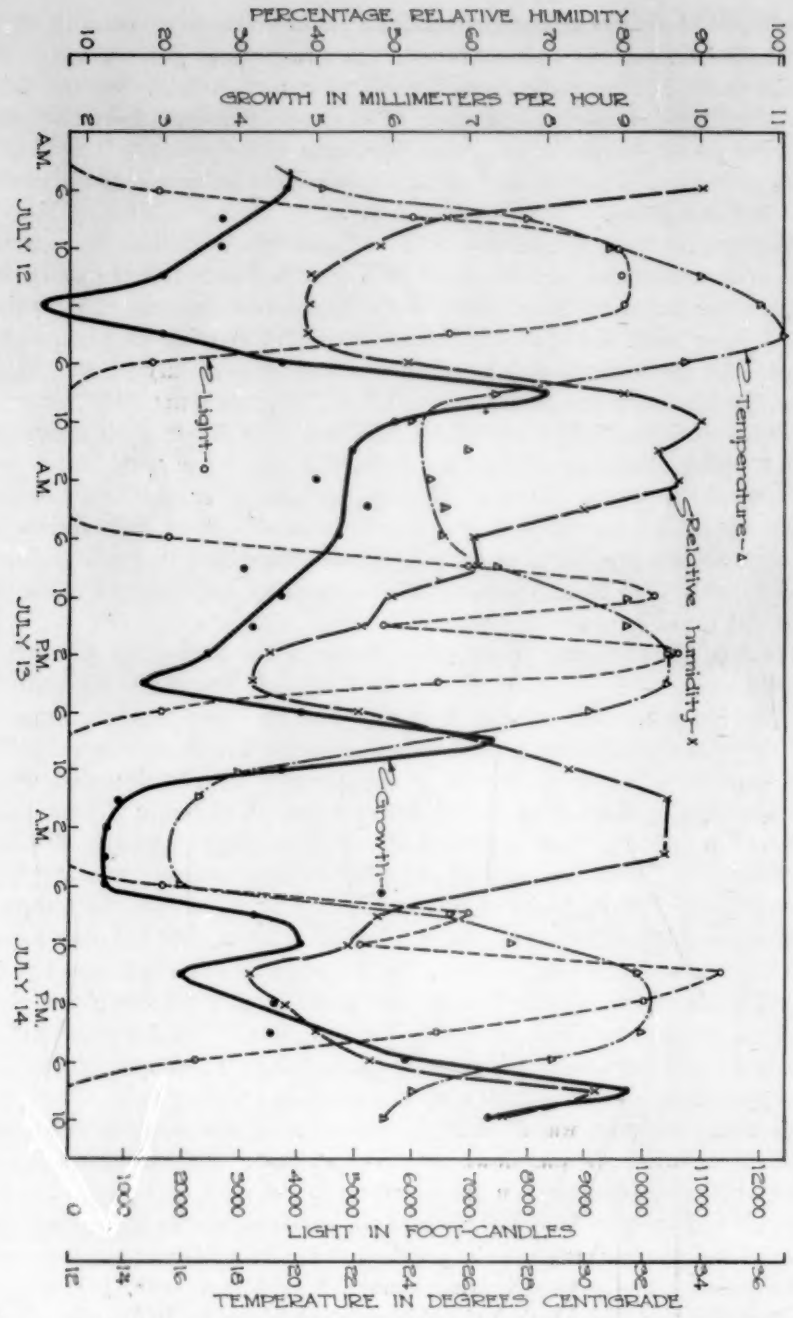


FIG. 5. Daily growth of maize with varying temperatures and deficient soil moisture.

Experiments with other plants

The data obtained with *Zea mays* seem clear cut. At temperatures between 10° to 15° and 30° to 35° C. the normal height growth of this plant was determined by temperature and internal moisture supplies. Light of the qualities and intensities obtained in Iowa sunlight had no direct effect upon its growth, although the indirect effects through photosynthesis, temperature, and transpiration are of major importance. Because the growing regions of the maize plant are thoroughly shielded from the direct action of the shorter wave lengths of sunlight, other, more exposed, plant organs were measured in further tests of the effect of light on growth.

Asparagus.—The growing points of young shoots of asparagus (*A. officinalis*) are more nearly exposed to direct sunlight, although they too are

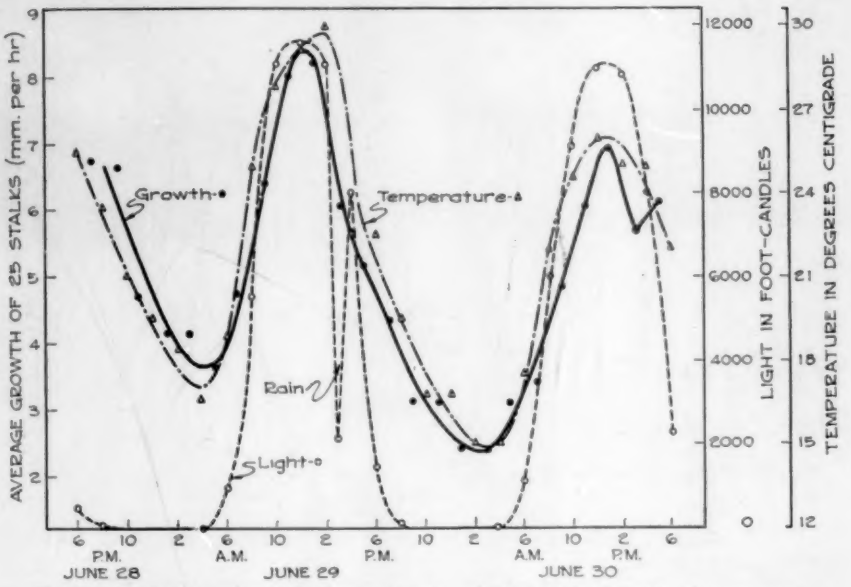


Fig. 6. Light intensity and temperature in relation to the growth of asparagus.

covered by layers of scale leaves. The young shoots differ from maize also in exposing very little transpiring surface. Stakes were driven by each of twenty-five actively growing young shoots of asparagus and their heights measured from this base at two-hour intervals over a 48-hour period. Average hourly growth rates, light intensities, and temperatures are plotted together in figure 6. The dependence of the growth of this plant upon temperature during this moderately warm, moist period is obvious from the data. These results agree with those of Culpepper and Moon (**4**) and Working (**23**). No inhibiting effect of light can be detected, and the growth of ten plants shaded with insulating board was not significantly different from that of the exposed plants.

Bindweed.—The growing points of the vine *Polygonum convolvulus* are only lightly covered by young leaves at the tips of stems about 1.0 mm. in

diameter. The meristems are thus well exposed to the direct effects of sunlight. Ten wild plants of this weed were marked and measured at four-hour intervals. Plants were some 80 cm. in length and just beginning to fruit at the start of the experiment. Although a full complement of leaves was exposed to full sun with slowly drying soil, Polygonum plants showed no evidence of an internal water deficit (fig. 7). Neither did they show any inhibiting effect of sunlight. Growth followed the temperature curve with the exception of the 2:00 to 6:00 P.M. jump on July 11. The four days shown were successively hotter and growth was successively less. Watering and shading, however, failed to restore the growth rate which continued to drop, apparently as a correlative effect of fruit production.

CASTOR BEAN.—The expansion of young leaves of *Ricinus communis* would seem to be a growth process particularly susceptible to inhibition by

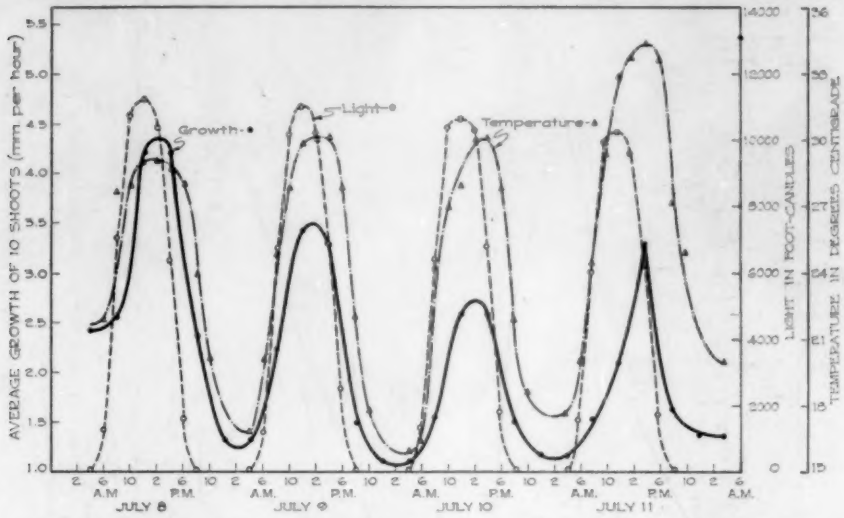


FIG. 7. Light intensity and temperature in relation to the growth of shoots of *Polygonum convolvulus*.

light. The thin blade tissue is fully exposed to any possible chemical as well as physical effects of sunlight, and the radiation might be expected to affect the formation, distribution, or action of growth hormones in the leaf (**1**), or possibly to act directly to speed up the differentiation processes (**10**) and thus check cell enlargement. The results shown in figure 8 are therefore somewhat surprising, for fully exposed leaves in bright July weather showed the same type of curves obtained with growing maize plants in June. Growth in width of these leaves rose with the temperature in the morning, dipped at midday with a water deficit, partially expressed by the relative humidity, rose again in the evening to drop again with the night temperature. Morning growth rates reached a maximum between 8:00 and 10:00 A.M. on July 23 (plotted at 9:00 A.M.) but 8 of 25 leaves made their greatest growth between 10:00 A.M. and 12:00 M. when the sunlight intensity ranged

from 10,000 to 11,000 fc. When allowance is made for the effects of decreased turgor in the expanding cells, it hardly seems possible to assign any additional inhibiting effect to sunlight.

One point in this experiment may be of special interest. With a random sample of young leaves large enough for their maximum width to be measured conveniently, and with growth rates of 2 or 3 mm. an hour, final leaf sizes ranged up to 40 cm. "Growth" in these large leaves, and possibly in all this material, was probably due to cell enlargement alone. There was no indication that growth rates in the larger leaves were quantitatively or qualitatively different from those in leaves of less than 10 cm. Cell enlargement generally is considered to be a physical process of water absorption, and as such should have a temperature coefficient of 1+. Instead, the coeffi-

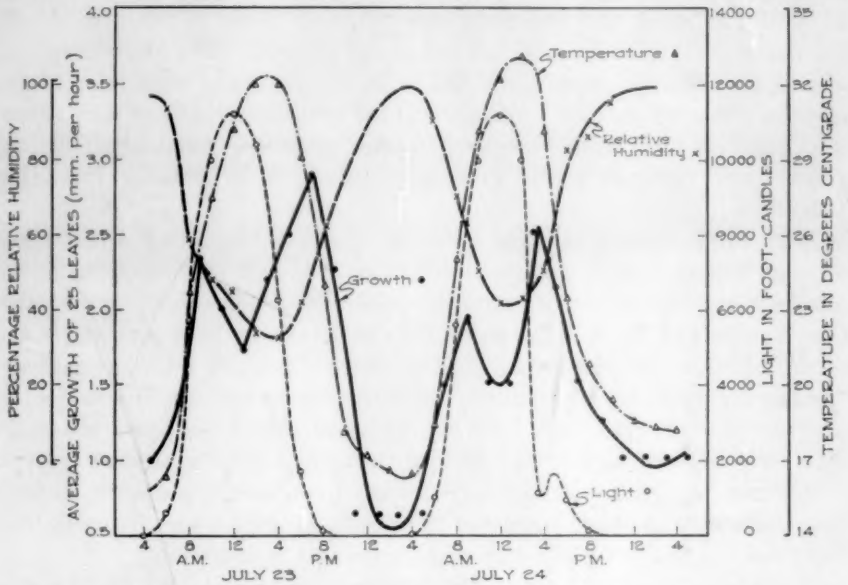


FIG. 8. Light intensity, temperature and relative humidity in relation to the expansion of leaves of *Ricinus communis*.

cients were very clearly 2+. Such results might be explained by assuming that the chemical formation and action of auxins in increasing cell wall plasticity is the major limiting factor in cell expansion, and that water supplies and turgor pressure are only secondary. If temperature is effective upon both cell division and cell enlargement, the close dependence of size increases upon temperature, shown throughout these experiments, is more easily explained.

Summary

Measurements of the combined elongation of the central axis and a young leaf of *Zea mays*, of the axis alone of *Asparagus officinalis* and *Polygonum convolvulus*, and of leaf blade expansion in *Ricinus communis* were made on field grown plants. In most of these plants the combined effects of cell

division and enlargement were included in the "growth" measurements, but in the larger leaves of the castor bean it is probable that cell expansion alone was concerned.

All measurements of both axes and leaves showed a tendency for size increases to follow the temperature curve, and thus, other factors being equal, to be *increased* in sunlight. Growth was checked, however, by water deficits within the plant. Such deficits are generally inversely proportional to the relative humidity and the available soil moisture, and directly proportional to light intensity, temperature, and air movement.

In numerous individual measurements maximum growth of both axes and leaves was obtained with full exposure to sunlight at 10,000 fc or more. Readings were made with a Weston photocell, and intensities as high as 12,000 fc, obtained with clear sun and reflecting banks of cumulus clouds, did not appear to be directly inhibiting for growth. No measurements of ultraviolet radiation were made, but, with the generally high relative humidities (50 to 60 per cent.) prevalent at the time these readings were taken, it is probable that values for this fraction were low, particularly in the shorter wave lengths which have been shown to be directly inhibiting (**12, 16**).

Full Iowa summer sunlight does not appear to have any direct effect upon growth of plants. Indirectly it increased growth by increasing photosynthesis and raising the temperature. Growth was decreased by excessive temperature and by internal water deficits developed in plants under the combined effects of sunlight, high absorptive capacity of the air and decreasing soil moisture percentages. Field grown plants were less subject to internal water deficits with high soil moisture than greenhouse plants in pots. The roots of field plants probably develop a greater absorptive area. Water near the plants was used preferentially, and sharp daytime checks in growth were observed when the principal absorption was occurring two feet from the base of plants with roots three feet or more long.

Experiments indicating that the expansion of large leaves has the temperature coefficient of a chemical reaction suggest that the action of auxins in increasing the plasticity of expanding cells may be more important than turgor pressure in cell enlargement.

The plants investigated made a greater growth in the daytime when temperature was the limiting factor, and a greater growth at night when moisture was limiting. Very commonly the interaction of these two factors produced a double peak of early morning and evening growth.

IOWA STATE COLLEGE
AMES, IOWA

LITERATURE CITED

1. AVERY, G. S. Jr. Differential distribution of a phytohormone in the developing leaf of Nicotiana, and its relation to polarized growth. Bull. Torrey Bot. Club **62**: 313–330. 1935.

2. BROWN, W. H., and TRELEASE, S. F. Alternate shrinkage and elongation of growing stems of *Cestrum nocturnum*. Philippine Jour. Sci. **13C**: 353–360. 1918.
3. COSTER, C. H. Die täglichen Schwankungen des Längenzuwachses in den Tropen. Rec. Trav. Bot. néerl. **24**: 257–306. 1927.
4. CULPEPPER, C. W., and MOON, H. H. Effect of temperature upon the rate of elongation of the stems of asparagus grown under field conditions. Plant Physiol. **14**: 255–270. 1939.
5. DAVIS, CHARLES H. Absorption of soil moisture by maize roots. Bot. Gaz. **101**: 791–805. 1940.
6. ————. Response of *Cyperus rotundus* L. to five moisture levels. Plant Physiol. **17**: 311–316. 1942.
7. INGE, F. D., and LOOMIS, W. E. Growth of the first internode of the epicotyl in maize seedlings. Amer. Jour. Bot. **24**: 542–547. 1937.
8. LEHENBAUER, P. A. Growth of maize seedlings in relation to temperature. Physiol. Res. **1**: 247–288. 1914.
9. LLOYD, F. E. Growth of Eriogonum in relation to light, temperature and transpiration. Carnegie Inst. Washington Yearbk. **15**: 58–59. 1916.
10. LOOMIS, W. E. Growth-differentiation balance vs. carbohydrate-nitrogen ratio. Proc. Amer. Soc. Hort. Sci. **29**: 240–245. 1932.
11. ————. Daily growth of maize. Amer. Jour. Bot. **21**: 1–6. 1934.
12. MASON, S. C. The inhibitive effect of direct sunlight on the growth of the date palm. Jour. Agr. Res. **31**: 455–468. 1925.
13. MCCALLA, A. G., WEIR, J. R., and NEATBY, K. W. Effects of temperature and sunlight on the rate of elongation of stems of maize and gladiolus. Canadian Jour. Res. **17**: 388–409. 1939.
14. OSMASTON, B. B. Rate of growth of bamboos. Indian Forester **44**: 52–57. 1918.
15. POPP, H. W. A physiological study of the effect of light of various ranges of wave length on the growth of plants. Amer. Jour. Bot. **13**: 706–736. 1926.
16. ————, and BROWN, FLORENCE. A review of recent work on the effect of ultraviolet radiation upon seed plants. Bull. Torrey Bot. Club **60**: 161–210. 1933.
17. PORTERFIELD, W. M. Jr. A study of the grand period of growth in bamboo. Bull. Torrey Bot. Club **55**: 327–405. 1928.
18. PRANTL, K. Ueber den Einfluss des Lichts auf das Wachsthum der Blätter. Arb. Bot. Inst. Wurzburg **1**: 371–384. 1873.
19. PRESCOTT, J. A. Some observations on the growth of maize in Egypt. Sultanic Agr. Soc. Bull. **7**: 1–25. 1921.
20. SACHS, JULIUS VON. Ueber den Einfluss der Lufttemperatur und des Tageslichts auf die stündlichen und täglichen Aenderungen des

Längenwachsthums (Streckung) der Internodien. Arb. Bot. Inst. Wurtzburg **1**: 99–192. 1872.

21. Trelease, S. F. Night and day rates of elongation of banana leaves. Philippine Jour. Sci. **23**: 85–96. 1923.
22. Went, F. W. Effects of light on stem and leaf growth. Amer. Jour. Bot. **28**: 83–95. 1941.
23. Working, E. B. Physical and chemical factors in the growth of asparagus. Arizona Agr. Exp. Sta. Tech. Bull. 5. 86–124. 1924.

FRUIT GROWTH AND FOOD TRANSPORT IN CUCURBITS

A. S. Crafts and O. A. Lorenz

Recently the writers reported that phloem exudation from cucurbits can no longer be considered a manifestation of normal food movement by mass flow. This does not mean that the growth of cucurbit fruits will not serve as an index of normal food movements, for only upon cutting the plant are these processes disturbed. The cultivated cucurbits are convenient to work with and grow very rapidly, so that the rates noted should represent the upper limits occurring in plants.

In 1941, Colwell (1) collected fruits of Connecticut Field pumpkins of five different sizes. From measurements and records he calculated the time required to grow from one size class to another. Table I gives the sizes of these fruits.

Colwell (1) secured further data on these fruits. In his own words: "For each size of fruit, measurements were made of the total cross-sectional area of the fruit stalk, of its total phloem, and of its total sieve tubes. The first two values were determined by mounting a fresh cross-section of the fruit stalk in water on a slide and projecting its image onto a large piece of white cardboard at a magnification of 25 diameters by means of a microprojector. The outline of the phloem groups and of the entire section was lightly traced on the cardboard in pencil. The traced image of the whole fruit stalk was cut out of the cardboard and weighed, after which the same thing was done for the individual phloem groups. A 100-sq. cm. area of cardboard was also weighed, permitting a calculation of the actual area represented by these groups.

"The total cross-sectional area of the sieve tubes of the fruit stalk was similarly determined by tracing all the sieve tubes from portions of phloem tissue of the fruit stalk onto white cardboard by means of camera-lucida and calculating from weights of cardboard the percentage of phloem tissue occupied by sieve tubes. The average of 10 such determinations indicated that 20.6 ± 5.2 per cent. of the total phloem is occupied by sieve tubes."

Reference here is to sieve-tube lumina. Table I includes the results of these measurements.

Knowing the dry weight increments and time periods between the size classes and having the measurements recorded in table I, rates of food transport required for the observed rates of growth may be calculated. Since the phloem exudate is not characteristic of the assimilate stream in cucurbits (4) and since there is no other basis on which to calculate concentrations more rationally, rates are given in terms of the total volume of sieve-tube lumina that would be occupied by the increment of dry weight being moved per unit of time. Because the sieve tubes are obviously never filled with dry food materials, these values must be multiplied by some factor representing the proportion of the sieve-tube-lumina volume occupied by foods in transport. Table II presents the data and calculations.

TABLE I

CROSS-SECTIONAL AREAS OF TOTAL PHLOEM AND OF SIEVE TUBES IN SINGLE FRUIT STALKS OF CONNECTICUT FIELD PUMPKIN (COLWELL'S DATA)

	FRUIT SIZE (ARBITRARY)				
	1	2	3*	4	5
Average fresh weight, grams	40	450	1,600	6,000	10,000
Total cross-sectional area of fruit stalk, sq. cm.	2.40	4.81	3.46	4.88	5.00
Total cross-sectional area of phloem groups, sq. cm.	0.125	0.154	0.128	0.255	0.499
Total cross-sectional area of sieve-tube lumina, sq. cm.	0.025	0.031	0.026	0.051	0.100

* The fruit stalk used for size 3 was small for the size class; the other values seem typical.

Judging from these calculations of COLWELL's data, the fruits studied grew rapidly and food transport must have proceeded at speeds difficult to conceive in view of the known structure of the phloem. For instance, during growth from size 1 to 5, foods moving by mass flow as a 20 per cent. solution would have to traverse the sieve tubes at a linear rate of around 80 cm. per hour; as a 10 per cent. solution, at 160 cm. per hour. If the foods move by diffusion on or along the parietal protoplasmic layer and through the sieve pores via the protoplasmic strands that traverse them, the rates would necessarily lie between 800 and 1600 cm. per hour.

To obtain more reliable data on growth rates and food transport, fruits of Connecticut Field pumpkin and Early Prolific Straightneck summer squash were measured while small, then marked and remeasured at intervals during the summer of 1942 by the writers. Finally they were harvested and weighed, and the areas of phloem in the fruit stalks were measured by the method described above. Table III presents the resulting data.

TABLE II

CALCULATED RATES OF FOOD TRANSPORT THROUGH THE SIEVE TUBES OF CONNECTICUT FIELD PUMPKIN (COLWELL'S DATA. CALCULATIONS BY THE WRITERS)

GROWTH PERIOD—FROM	SIZE 1 TO 2	SIZE 2 TO 3	SIZE 3 TO 4	SIZE 4 TO 5	SIZE 1 TO 5
Length of growth period, hours	96.0	120.0	168.0	288.0	672.0
Total dry weight increment, grams	22.8	65.5	287.0	432.0	807.3
Dry weight increment, grams per hour	0.237	0.546	1.71	1.50	1.20
Dry weight increment ml. per hour*	0.158	0.364	1.14	1.00	0.80
Average area of sieve-tube lumina, sq. cm.	0.028	0.026	0.036	0.075	0.050
Linear rate of displacement, cm. per hour	5.64	14.00	31.67	13.33	16.00

* Based on the assumpton that the average specific gravity of the pure dry food materials is 1.5.

In order to use the values in table III for calculating rates, one must establish the relation between the linear dimensions of the fruits and their

TABLE III

DIMENSIONS, FRESH WEIGHT, AND FRUIT-STALK PHLOEM AREA OF CONNECTICUT FIELD PUMPKINS, 1942

FRUIT NUMBER	AUGUST 5		AUGUST 25		SEPTEMBER 7, HARVESTED				
	LENGTH	WIDTH	LENGTH	WIDTH	LENGTH	WIDTH	WEIGHT	PEDUNCLE AREA	PHLOEM AREA
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*kgm.*	*sq. cm.*	*sq. cm.*
1	5.3	4.8	17	21	17	22	2.61	1.52	0.129
2	2.6	2.4	.....	.....	19	25	3.96	2.28	0.266
3	5.1	4.2	22	24	24	26	5.88	1.69	0.142
4	2.9	2.3	.....	.....	21	24	4.07	2.79	0.279
5	5.0	4.5	24	28	25	37	7.84	2.58	0.246
6	11.4	10.1	28	29	28	30	8.17	2.44	0.358
7	10.4	9.8	19	23	21	27	4.99	1.95	0.231
8	2.8	2.3	20	27	22	28	6.68	1.52	0.097
9	3.5	3.1	21	25	21	25	4.54	2.02	0.149
10	2.9	2.4	.....	.....	36	38	7.49	2.30	0.218
11	2.2	2.1	.....	.....	24	34	9.85	2.66	0.159
12	3.4	2.7	.....	.....	15	16	1.59	1.61	0.124
13	4.2	3.3	16	17	16	17	1.70	1.87	0.226
14	6.3	5.5	.....	.....	23	33	8.51	1.84	0.137
15	3.6	2.7	.....	.....	34	32	4.76	1.72	0.089
16	6.9	5.8	25	29	26	29	7.93	2.66	0.289
17	2.6	2.3	21	20	25	27	6.90	1.81	0.113
18	5.2	3.6	23	23	24	23	4.65	3.07	0.227
19	3.6	2.5	28	26	34	30	9.75	2.19	0.187
20	5.4	5.1	22	28	22	29	5.90	2.11	0.143
21	5.3	4.9	19	23	19	24	3.63	1.56	0.161
22	2.0	2.0	19	22	20	26	4.31	1.22	0.080
23	4.8	4.8	14	19	16	22	3.17	1.57	0.122
24	6.0	5.7	.....	.....	21	29	6.80	1.80	0.306
25	6.8	6.8	20	29	21	29	6.35	1.84	0.186
26	6.2	6.1	20	27	21	30	6.68	1.99	0.163
27	3.6	3.4	.....	.....	19	25	3.85	1.67	0.143
28	5.7	4.3	.....	.....	28	30	7.93	2.36	0.159
29	3.9	3.4	.....	.....	21	24	4.08	1.39	0.154
30	6.0	5.5	.....	.....	18	23	3.40	1.44	0.167
31	6.2	5.7	19	24	21	25	5.89	2.36	0.292
32	7.6	7.0	24	32	26	33	7.48	1.75	0.153
33	6.8	6.1	19	25	20	27	4.65	1.53	0.188
34	5.1	4.3	20	24	20	27	5.10	1.48	0.150
35	5.4	5.4	.....	.....	11	15	1.13	1.36	0.118
36	5.9	5.7	.....	.....	20	27	5.21	3.09	0.359
37	2.5	2.4	.....	.....	21	29	6.12	2.24	0.173
38	4.3	3.0	.....	.....	30	36	7.02	3.33	0.150
39	4.7	3.4	.....	.....	31	28	9.30	2.42	0.216
Total	194.1	171.4	460.0	545.0	881.0	1061.0	219.87	79.03	7.249
Average	4.98	4.40	20.9	24.8	22.6	27.2	5.64	2.03	0.186

fresh weights. For this purpose 28 small fruits of Connecticut Field pumpkin, covering the range in size represented by the August 5 measurements in table III, were measured and weighed.

Some of these small fruits were spherical; others somewhat cylindrical

with hemispherical ends. Their approximate volumes can be calculated by the following formula:

$$V = 4/3\pi r^3 + \pi r^2 (l - d) \quad (1)$$

where V = volume
r = radius
l = length
d = diameter

By use of this formula approximate volumes of the 28 fruits were calculated and a graph drawn representing the relation between volume and weight. Employing this graph and using approximate volumes calculated from linear dimensions one may determine the weight of any fruit, the dimensions of which come within the limits of those used in making the graph.

Fruit volumes so calculated have proved to be somewhat higher than actual volumes, measured by displacement. Since, however, the calculated volume is used only as a function of the linear dimensions and not in the determination of rates, the errors tend to compensate and they affect the rate values little, if any.

From table III, the fruits measured on August 5 averaged 4.98 cm. in length and 4.40 cm. in diameter. The average volume was 53.5 ml. Checking this volume against the curve mentioned above, the average fruit weighed about 51.5 gm. at the time of marking. The average weight at harvest time was $219.87 \div 39 \times 1000 = 5637.7$ gm. The gain therefore was $5637.7 - 51.5$ or 5586.2 gm. in the 33-day growth period. At the time of collecting, the average transverse phloem area was 0.186 sq. cm. per fruit stalk.

The fruits of table III were at about the same stage of maturity on September 7 as those reported to be size 4 in table V of our previous paper (**4**). On an average, they were somewhat smaller because they had undergone no selection, whereas those mentioned in table V had been selected for uniformity at a definite size.

Fruits of size 4 [table V (**4**)], had a dry-weight composition of 8.65 per cent. Applying this figure to the average fresh-weight increment, the average dry weight should have been 5586.2×0.0865, or 482 gm. This weight divided by 792, the number of hours in the growth period, gives the average hourly dry weight increment that moved into these fruits. This value, 0.61 gm., is the average amount of dry material that moved into the fruit each hour between August 5 and September 7. Based on a specific gravity of 1.5, this hourly dry weight increment would occupy 0.41 ml.

According to several studies (**1, 2, 3, 6**), an average value for the proportion of the total phloem occupied by the sieve-tube lumina is 20 per cent. Using this figure, the average phloem value for the peduncles of fruits of table III would be $0.186 \times 0.2 = 0.0372$ sq. cm. Dividing this area into the volume representing the hourly dry increment of food transported into the pumpkin fruits, the linear displacement rate would be $0.41 \div 0.0372$, or 11.0 cm. per hour. This is reasonably close to the value of 16.0 found in table II from COLWELL's data.

The value given above is an average. Calculating similar values for the three largest fruits and for the three smallest, we obtain the following values: for the largest fruits 21, 17, and 14 cm. per hour; for the three smallest 4, 3, and 3 cm. per hour.

These are, of course, lower than any possible real values: they represent rates at which pure dry food materials would have to move in order to provide for the observed rates of growth. If this food material were moving as a 20 per cent. solution, the rates would necessarily be five times the values given above; if it moved as a 10 per cent. solution, they would be ten times as great; and if it moved as individual molecules, independent of water but associated with the cytoplasm, the values must be multiplied by a factor between 50 and 100. The rates given above represent, furthermore, averages for the total growth period. According to COLWELL's data as given in table II, the rate during the most active growth period may be approximately twice the average rate for the whole period of growth. And even this rate does not take into account the diurnal fluctuations, which may be appreciable.

All the fruits reported so far were green during most of their period of growth. Photosynthesis is an unknown factor in these calculations, as is respiration. To avoid the error from photosynthesis, measurements were made on Early Prolific Straightneck squash, whose fruits are cream-colored when very young and yellow when mature. Respiration was not measured, but the error from it results in underestimation of rates rather than overestimation. Values given in the following pages are, accordingly, on the conservative side.

Following the previous experiment, fruits of Early Prolific Straightneck squash were tagged and measured periodically and were finally harvested, measured, and weighed. To evaluate the dimension measurements it was again necessary to establish the weight-volume relation. This was done by collecting 75 squashes, ranging in size from those of recently pollinated flowers to mature fruits, measuring and weighing them, and plotting volume against weight.

Thirty-six small fruits were measured and tagged on September 7, measured on September 10 and 15, and harvested on September 18, 1942. The final harvested weights were obtained directly by weighing; the others were taken from the graph mentioned above, using the volumes calculated by equation 1. Areas of fruit stalks and phloem were obtained by projecting sections and weighing the outlined areas, as described earlier.

Averages for growth during the three measured periods were obtained by subtracting the initial from the final weight values for the periods. These averages were 204.6, 477.8, and 186.8 gm. for the three periods, and 869.3 gm. for the total growth period. Dividing each by the number of days in the period, the average daily growth rates were 68.2, 95.5, and 62.2 gm. for the three periods, and 79.0 gm. for the total period.

To give a clearer view of the growth rates of the individual fruits, table IV lists the increments for the three periods measured and for the total

growth period, both on the period basis and on the daily basis. Finally, it gives a series of values for rate of transport that were obtained by dividing the average daily growth increment for the total period by 24 (to put it on an hourly basis) and by the area of the sieve-tube lumina or one-fifth of the phloem area. These values represent the linear rate, in centimeters per

TABLE IV

GROWTH INCREMENTS OF EARLY PROLIFIC STRAIGHTNECK SUMMER SQUASH FOR THREE GROWTH PERIODS

	SEPT. 7–10 INCREMENT		SEPT. 10–15 INCREMENT		SEPT. 15–18 INCREMENT		TOTAL PERIOD INCREMENT		TRANSPORT RATE
	TOTAL	DAILY	TOTAL	DAILY	TOTAL	DAILY	TOTAL	DAILY	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cm./hr.*
1	248	82.8	295	59.0	23	7.8	567	51.6	186.5
2	350	116.7	208	41.6	77	25.7	635	57.7	148.5
3	346	115.5	605	121.0	67	22.3	1018	92.6	268.0
4	295	98.3	379	75.8	108	36.0	782	71.1	214.5
5	361	120.3	167	33.4	48	16.2	576	52.4	147.5
6	376	125.3	598	119.6	141	47.0	1115	101.4	306.0
7	248	82.7	368	73.6	47	15.8	663	60.3	157.5
8	144	48.0	215	43.0	32	10.7	381	34.6	85.0
9	414	104.8	624	124.8	86	28.8	1125	102.3	339.0
10	308	102.8	521	104.2	137	45.8	958	87.2	204.0
11	175	58.3	371	74.2	134	44.7	680	61.8	176.5
12	229	76.3	456	91.2	167	55.7	854	77.6	294.0
13	83	27.9	377	75.4	203	67.8	664	60.4	163.5
14	162	54.0	389	77.8	129	43.0	680	61.8	149.5
15	63	21.2	493	98.6	151	50.5	708	64.4	206.5
16	55	18.6	431	86.2	206	68.8	693	63.0	193.0
17	104	34.8	670	134.0	498	166.0	1272	115.7	238.5
18	67	22.3	519	103.8	452	150.7	1038	94.4	293.5
19	128	42.9	510	102.0	189	63.0	827	75.2	245.0
20	64	21.4	585	117.0	196	65.3	687	62.5	200.5
21	227	75.7	569	113.8	270	90.2	1066	97.0	243.5
22	194	64.7	806	161.2	228	76.2	1228	111.7	280.5
23	422	107.3	717	143.4	74	24.8	1213	110.3	377.0
24	375	125.0	415	83.0	96	32.0	886	80.5	207.0
25	348	116.1	883	176.6	117	39.0	1348	122.6	327.5
26	252	84.0	529	105.8	306	102.0	1087	98.8	260.5
27	66	22.1	384	77.0	292	97.5	743	67.6	168.0
28	140	46.9	510	102.0	283	94.5	934	84.9	203.5
29	117	39.0	589	117.8	231	77.0	937	85.2	219.0
30	108	36.0	673	134.6	424	108.2	1205	109.6	251.0
31	214	71.5	427	85.4	64	21.5	706	64.2	239.0
32	143	47.8	351	70.2	29	9.8	523	47.6	171.0
33	264	88.3	457	91.4	142	47.5	864	78.6	202.5
34	79	26.5	408	81.6	320	106.8	808	73.5	194.0
35	123	41.2	379	75.8	284	94.7	786	71.5	204.0
36	64	21.3	488	97.6	467	155.7	1019	92.6	182.0

hour, at which a solution equivalent in dry-weight concentration to the larger fruits [table III, (**4**)] must flow through the sieve-tube lumina to provide for the observed rates of growth. They range from 85.0 to 377.0 cm. and average 268.5 cm. These rates are based on a dry-weight composition of about 5 per cent. On a pure dry-weight basis this average value would be 13.4 cm. as compared with our figure of 11.0 for Connecticut Field pumpkin and COLWELL's figure of 16.0 for the same species. If this food were

moving as a 10 per cent. solution, the rate would be around 130 cm. per hour. If it moved by diffusion along the protoplasm, the rate would be in the range of 650 to 1300 cm. per hour.

Discussion

Many rates of food movement in plants have been given in the literature. Since the mechanism of movement is as yet unknown, all such values are calculated or estimated by methods like those used above or by measurements on viruses, radioactive elements, fluorescent dyes, or other compounds assumed to be moving in the phloem but not serving as foods in the plant. Though chemical analyses on growing fruits provide reliable data on the daily or hourly amounts of dry weight added, the bases for transforming these to linear rates are only hypothetical: no one has yet determined whether the foods are moving through the total phloem, through the sieve-tube lumina, or along the protoplasm.

If foods move in plants by a mass flow of solution, the rate values found in this work must all be multiplied by a factor representing the quotient of the solution volume divided by the solute volume. The smallest probable value for such a quotient would be around 5, actual values might range to 10 or more. Using such factors on the rate values found, actual linear transport rates for foods in solution would lie between 55 and 160 cm. per hour for these species.

If foods move by an activated diffusion process, they must of necessity be closely associated with the cytoplasm of the sieve tubes. According to present day concepts of sieve-tube anatomy (**5**), the cytoplasm of the mature element occupies a very small portion of the space within the walls; it is a thin parietal layer lining the walls and surrounding the large central lumen. Providing this picture of sieve-tube structure is accurate, then the rate values found in this work must all be multiplied by a factor made up of the reciprocal of the proportion of the sieve tube occupied by cytoplasm times the reciprocal of the proportion of the cytoplasm occupied by food materials. Estimates of cytoplasmic volume of mature sieve tubes lie between 1 and 2 per cent. of the transverse area; it does not seem probable that foods would occupy over 20 per cent. of the cytoplasm during their movement through or along it. Such estimates indicate that the factor to be applied to the rates calculated in this work should be of the order of 100 or more. Such being the case, actual linear rates of movement must lie around or above 1000 cm. per hour during rapid transport of foods.

The above calculations are only approximate and are designed to provide some idea of the order of magnitude of transport rates required to account for growth and storage in highly active plants. Though the measurements leave much to be desired, they do provide a fairly reliable basis for arriving at approximate values for translocation rates. Enough fruits were measured to give dependable average values; measurements were taken often enough to catch the high rates that occur only for short periods; and

analyses were made to show the amounts of the principal food constituents that were being moved [tables III and IV (**4**)].

The rates calculated from the measurements are obviously high, for the material used was chosen to indicate the upper limits of transport velocity. Such studies yield little evidence of the type of mechanism involved. But any mechanism proposed to account for food transport must be compatible with the observed velocities if it is to care adequately for normal growth rates in vigorous plants.

Summary

Calculations based on COLWELL's data prove that dry food materials would have to pass through the sieve-tube lumina of Connecticut Field pumpkin peduncles at an average rate of 16 cm. per hour in order to account for fruit growth. For a seven-day period the rate was over 31 cm. per hour.

Similar calculations based upon many measurements of fruit and phloem areas indicate transport rates of 11.0 and 13.4 cm. per hour for Connecticut Field pumpkin and Early Prolific Straightneck squash respectively.

Such rate values, when converted to linear displacement values for solutions, indicate movement at rates of 55 to 160 cm. per hour for transport by mass flow. By activated diffusion along the cytoplasm the above rate values indicate diffusion rates of 1000 cm. per hour or above.

UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA

LITERATURE CITED

1. COLWELL, R. N. Translocation in plants with special reference to the mechanism of phloem transport as indicated by studies on phloem exudation and on the movement of radioactive phosphorus. Thesis, Univ. of California. 1942.
2. CRAFTS, A. S. Movement of organic materials in plants. Plant Physiol. **5**: 1–42. 1931.
3. ————————. Phloem anatomy, exudation, and transport of organic nutrients in cucurbits. Plant Physiol. **7**: 183–225. 1932.
4. ————————, and LORENZ, O. A. Composition of fruits and phloem exudate of cucurbits. In press.
5. ESAU, KATHERINE. Development and structure of the phloem tissue. Bot. Rev. **5**: 373–432. 1939.
6. MASON, T. G., and LEWIN, C. J. On the rate of carbohydrate transport in the greater yam, *Dioscorea alata* Linn. Roy. Dublin Soc. Proc. **8**: 203–205. 1926.

RELATION OF WEATHER CONDITIONS TO ONION BLAST[1]

LINUS H. JONES

(WITH TWO FIGURES)

Introduction

During the recent period including the years of 1925 to 1940 there have been seven years in which the crops from onion seed suffered a serious leaf injury resulting in low yields. The disease is apparently physiological, for there has not been a single instance in either past or recent investigations that showed the presence of a pathogen when the plants exhibited the first signs of injury.

The hypothesis that the causal agent is a certain combination of weather conditions has been substantiated by observation and also by laboratory experiments simulating the environmental factors that approximate the weather conditions prevalent preceding and during the appearance of the injury.

Onion blast symptoms

The symptoms that lead to the diagnosis of the malady as onion blast are so evident and appear so suddenly that the word "blast" has been used in place of the milder term sunscald. In extreme cases, the tops of the leaves will turn white in a few hours, the course of the injury being from the tip downward. As the whitened part later turns brown, the remainder of the leaf dies gradually over a period of a few days, the rate becoming slower in cloudy weather. The most extreme symptom that has been observed is the death of the leaf tip without the immediate destruction of the chloroplasts, the tip turning to a dark green lacking the whitening that occurs in a less sudden death. A milder and more common form of blast symptom is the appearance of white spots on the leaves (around the stomata) which gradually enlarge as the leaf dies back from the tip. If the weather during the onset of blast remains fair, considerable damage results. If, however, cloudy weather occurs soon after blast symptoms appear, the plants recover to the extent that they seem to adapt themselves to conditions which allow a balance between the amount of water absorbed and that lost by transpiration.

Environmental factors

As previously implied, the term blast indicates the suddenness of the appearance of the disease. Onion blast has always been observed to coincide with the occurrence of a few hours of clear, dry weather following a prolonged period of cloudy weather accompanied by rains. The injury results from a maladjustment to the environment of high light intensity (sunshine) following the prolonged period of low light intensity. These two environments may be described, in general, as follows.

[1] Contribution no. 436 of the Massachusetts Agricultural Experiment Station.

First environment: Subdued light, warm air, and high humidity with a very moist soil at a time when the plant is actively growing and bulb formation is incomplete. This environment is possible over an extended period of cloudy weather with much rain. In such an environment, the onion leaves grow rapidly and have a soft texture. The plants are shallow rooted and the root system is small in relation to the total area of leaf surface. Since the cloudy, humid weather does not favor a heavy loss of water by transpiration, a small root system in a very moist soil is adequate for supplying the needed moisture to the plant. This first environment sets the stage and injury will occur when the second environment appears.

Second environment: Bright sunshine, high temperature, low humidity. The immediate effect on the onion plant is increased rate of transpiration, the rate being so rapid that more water is lost than can be absorbed by the limited root system. Transpiration at the lower portions of the leaves so depletes them of moisture that turgor is much reduced and the upper portions become flaccid. The tip portions become whitened because of their parched condition and the bleaching action of full sunlight. White areas occur around the stomata because water is lost at these points on the leaves more rapidly than the limited supply can replace it. The loss from the stomata is so rapid that water is also withdrawn from the adjacent cells, with the result that these cells are killed. Death of the cells at the tips and about the stomata so disrupts the normal function of the leaf that it dies back proportionately to the extent of the damage.

In general, it may be stated that extent of the damage is related to the modified or abnormal growth and to the ability of the air to withdraw moisture from such plants. Thus a root system which is small reduces the ability of the plant to replace lost moisture. Hence bright, warm, dry weather will cause excessive transpiration followed by tissue dehydration and occurrence of blast.

Observations and experimental work

A composite record of field observations gives the following general conditions that are associated with onion blast. The injury appears suddenly and is widely extended. It is noticed first in the presence of intense sunlight and high temperature. Any increase in the development of the injury is on individual plants and not from plant to plant, where there may be borderline conditions of injured and uninjured plants. The nature of the appearance of the injury—general instead of sporadic—the fact that it increases during bright weather, and the apparent lack of evidence of causal infection by a pathogen prior to injury all indicate that the disease is physiological and, therefore, not caused by a parasite.

Usually, the onset of blast has taken place in July, the middle of the month being considered the most critical period. While the weather conditions that precede blast may affect the quality of the crop from set onions, blast does not entirely ruin the crop since harvesting begins within the last ten days of the month. On the other hand, seed onions are not ready for

harvesting until the middle of August and the effect of blast on this crop is so completely disastrous that the seed onion crop may be a total failure.

In the laboratory, it has been possible to simulate the weather conditions believed to be the important factors concerned with governing the type of plant development that cannot withstand the sudden transition to the normal environment of dry, clear, and warm weather. Onion plants from seed were grown in 6-inch flower pots. When the bulbs were fairly well formed and still actively enlarging, the plants were deemed ready for use. To obtain the environment of subdued light and high humidity, a Wardian case was lined with paper and the bottom covered with a layer of moist sphagnum moss.

At various intervals, potted onion plants were placed in the prepared Wardian case and, as weather conditions warranted, these plants were moved to an out-of-door situation for the second environment (a fair, dry day and a high temperature). In no one experiment was this second environment as ideal as the conditions which prevailed in onion blast years. Check plants were grown in pots in the regular greenhouse environment and placed alongside those which were moved from the Wardian case. The soil was kept moist at all times, especial care being taken that when the plants were subjected to the second environment, water was added to the soil of both the test and check plants.

A period of no longer than 10 to 14 days was sufficient to so weaken the plants in the Wardian case that they suffered tremendously when exposed to the brighter and less humid conditions of the second environment. When these plants were exposed to strong sunlight on a day of low humidity, the first effect on the plants was extreme wilting. Check plants remained upright. Other results of the effect of exposure were not always the same. There were times when the surface of the leaf assumed a grayish-white color within a few hours. Most frequently it was the tips of the leaves that whitened and these leaves died and became brown in the course of a few days. The tips, in most instances, died first and the browning area gradually descended the whole leaf. In some tests the leaf tips puckered or crinkled and became dry without any appearance of the blanching symptoms associated with sunscald. They remained a dark green for several days after which the chlorophyll disappeared leaving the tips brown. That this form of injury was related to lack of water in the leaf was demonstrated by severing the root system from check plants. The same type of puckering injury resulted, indicating that sunscald, or onion blast, is finally brought about by the dehydration of plant tissues which lose more water to the air than the root systems can supply.

Frequently, when the plants wilted and the upper portions of the leaves fell at an angle because of lack of turgor, the upper side whitened due to exposure to the direct rays of the sun. The lower side remained temporarily green but eventually became brown, the damage being sufficient to kill even the area protected from direct exposure (fig. 1).

A comparative study was made of the plants placed in the Wardian case for 16 days and the plants grown in the greenhouse. The subdued light and high humidity caused the leaves to be slightly smaller than those of the checks. There were more chloroplasts in the tissue of the leaves which had

FIG. 1. Sunscald (blast) of onion leaves.

not been subjected to the subdued light. There was no difference in the size of the stomata or the number per unit area of leaf surface in the two sets of plants grown under poor and good light conditions. There was, however, a marked contrast in the size of the root systems, those of the plants grown under subdued light and high humidity being the smaller.

Observations made in the field during onion blast years also show a small root system. It seems, therefore, quite logical to conclude that when more water is transpired from the plants than the limited root systems can replace, water is gradually withdrawn from the tissues in response to the evaporating power of the air, and the resulting dehydration of the tissues shows itself as sunscald in the laboratory and as onion blast in the field.

Relationship of prolonged cloudiness, relative humidity, and hours of sunshine to onion blast

A study of official weather records (**4**) obtained from the region where onion blast has been observed, provided a source of data that proved quite

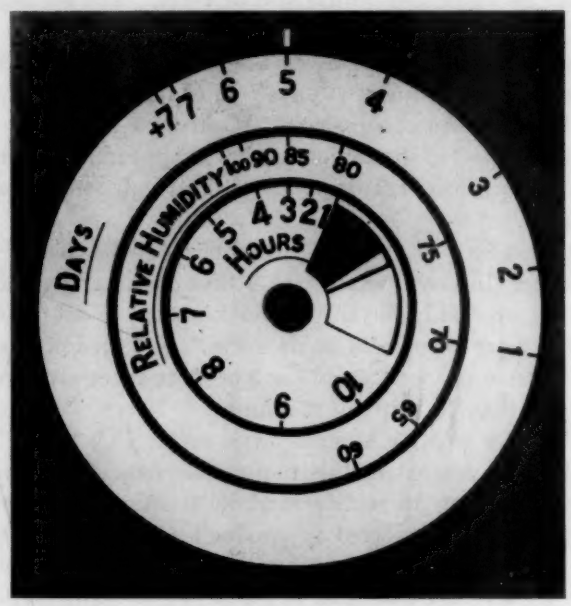


Fig. 2. Dial chart. The combination of dials may be constructed of heavy paper or light weight cardboard by drawing perpendicular intersecting lines and using the point of intersection as the center for each circle.

useful. Such weather records have limitations in that continuous data for a factor are reported as maximum, minimum, mean, or average. In this study of the elements of weather there seemed to be certain facts related to onion blast that could be shown visually. Graphical representations using two or more of these factors led to confusion instead of clarity. The thought of superimposing the values of one factor over the values of another seemed plausible and thus originated the dial chart. By such superimposed dials it is possible to group the weather conditions to which plants and their parasites respond. This may be done in terms of beneficial or detrimental effects on the plant. In all cases, both good and bad influences are present and the dial chart may be used to express and make visual the combination

of forces, good or bad, which is likely to prevail. Thus, if the chart is developed to show on its colored background the element of sensitivity to disaster, a lack of color showing from the data at hand would indicate that one or more beneficial elements are sufficiently strong to offset detrimental elements.

In order to present visually and more specifically the range of combinations of number of cloudy days, average relative humidity, and average number of hours of sunshine that precede blast, a dial chart was constructed (fig. 2). The background card should have the upper right quarter colored (crayon). In the dials, complete areas are cut from the upper right quarter, these areas being equidistant from the center and equal to each other. The whole may be assembled with a pin or rivet. The setting point is at top center of background.

The above dial chart is so arranged that the elements of weather can be combined in terms of cloudiness ("days" on chart), relative humidity, and average number of hours of sunshine. If an area is shown on the background card it is expected that onion blast will appear in the field when the weather changes to a bright, sunny day with a high temperature and low relative humidity.

Judgment must be used in setting the limits on the dials. Lower limit of cloudy weather, in days, was set at 3 thus making the value 4 as the minimum period of cloudy days. The maximum was set at +7 as it was judged that 8 or more days could be no worse. A period of cloudy weather was determined from weather records as 3 or more successive days, each day of which had less than 10 hours of sunshine.

The low limit for relative humidity was set at 75. Since 70 is normal for June, July, and August in this region, the figure 75 is not excessive. Eighty is the first figure to register and its position is such that it combines with other factors involved in producing a soft, abnormal development of the onion plant. The maximum of 100 is, of course, obvious.

The limits for average hours of sunshine were again matters of judgment, 6 hours being deemed sufficient to prevent excessive growth and softness of tissue.

It will be noted that the position of the figures is such that they are closer together as maximum detrimental conditions are involved.

Lower limits of number of days of cloudy weather (set at 3) and percentage of relative humidity (set at 75) and an upper limit of average hours of sunshine (set at 6) were arbitrarily chosen.

In order to demonstrate by the dial chart that sensitizing the onion plant to blast is due to a combination of factors and not one factor, attention is directed to a comparison of items in table I. It will be noted that many of the periods of cloudy weather (three or more days in succession with less than 10 hours' daily sunshine) in non-blast years (items 18–27) are equal to and sometimes longer than cloudy periods of blast years (items 1–17). This comparison eliminates cloudy weather as the one and only factor.

Neither is relative humidity the deciding factor. In items 23, 24, and 27, where the average relative humidity figures are 80 in non-blast years, there are three periods in the blast years when the relative humidity was the same (items 7, 9, and 14).

A similar comparison can be made to show that the average number of hours of sunshine in the period is not the one factor that sensitizes the plants.

TABLE I

SAMPLES OF CLOUDY PERIODS IN BLAST AND NON-BLAST YEARS. LENGTH OF PERIOD IN DAYS WITH AVERAGE RELATIVE HUMIDITY AND AVERAGE HOURS OF SUNSHINE SHOW SIMILARITIES IN THE BLAST AND NON-BLAST YEARS BUT THE EFFECTS ON SENSITIZING THE PLANTS TO BLAST MAY BE DETERMINED BY THE DIAL CHART

Item	Year	Dates	Period	Average relative humidity	Average number hours of sunshine
			Blast years		
			days	*%*	*hours*
1	1927	5/9–5/27	19	90	2.34
2		7/12–7/23	12	95	5.88
3	1928	5/18–5/30	13	90	2.73
4		6/4–6/7	4	90	2.43
5		6/18–7/2	15	85	3.26
6	1931	6/7–6/12	6	85	1.31
7		7/6–7/12	7	80	3.95
8	1935	6/3–6/11	9	85	1.99
9		6/17–6/25	9	80	4.30
10		7/7–7/10	4	90	3.65
11	1937	6/18–6/23	6	85	3.70
12		6/26–7/3	8	85	3.69
13	1938	7/18–7/24	7	85	0.67
14	1940	5/1–5/4	4	80	2.12
15		5/20–5/29	10	85	2.63
16		6/8–6/13	6	90	3.43
17		7/20–7/26	7	90	4.94
			Non-blast years		
18	1929	6/18–6/25	8	70	4.51
19		6/28–7/10	13	75	5.10
20		7/21–7/29	9	70	7.30
21	1930	5/24–5/29	6	70	4.25
22	1932	6/16–6/22	7	75	5.83
23	1933	5/24–6/1	9	80	4.90
24		6/25–6/30	6	80	5.02
25	1934	7/24–7/31	8	75	4.20
26	1936	6/10–6/15	6	75	2.07
27	1939	7/25–7/31	7	80	4.73

Item 26 is low; 2.07 average hours of sunshine and 6 days of cloudy weather. Yet in blast years items 11 and 16 had equal periods of cloudy weather and more hours of average sunshine.

In general, a comparison will show that the blast year items have the combination of high relative humidity and a relatively lower value for average hours of sunshine.

The element of air temperature could have been included in the chart by adding another dial. It was, however, quite noticeable in the weather records that high temperatures usually prevailed when high relative humidity was present and low relative humidity values were accompanied by low temperatures during the cloudy periods. The extra dial for temperature would have added little to the value of the chart which should be as simple as possible.

The dial chart is not a measure of the intensity of blast effects. Blast follows when the onion plants have been sensitized by weather conditions. The degree of sunlight over an extended period of several hours coupled with low relative humidity and high temperature are the weather conditions that really bring on blast, once the plants have been sensitized. The amount of colored background on the dial chart, exposed by the setting of the dials according to weather records, gives a measure of the sensitivity of the onion plant to blast.

Discussion

WHETZEL (**5**), and CLINTON (**1, 2**) have published their observations in regard to this or a similar type of injury. WHETZEL refers to it as "white tips." DORAN and BOURNE (**3**) were able to delay the onset of blast by spraying with Bordeaux mixture. Although spraying did increase the yield in a blast year, these investigators concluded that the increase was not enough to justify spraying every year. Their observations on onion diseases and thrips damage pointed, in their opinion, to "a physiological rather than a parasitic cause of onion blast."

The fact that the injury develops and increases in bright sunshine and low humidity is in marked contrast to the inception and spread of parasitic diseases which depend upon cloudy days and moist air for an optimum environment. All observations point to, and laboratory experiments confirm, the cause of onion blast as the too rapid loss of water from sun-exposed tissue after the abnormal development of the plant in subdued light, high relative humidity, and high temperature.

In all probability weather records are rather more closely tied to man's relation with weather than to a complete understanding of the relations between plants and weather. Consequently, some guesswork is involved in translating weather records, as published, to a system useful for correlating plant responses. The idea of handling the elements of weather as superimposed dials must, to a great degree, include judgment and leeway. This type of chart should prove useful in balancing plant development with weather or other growth factors. It should also prove useful in forecasting the spread of disease from initial infections and give a fair opportunity to estimate the probable extent and seriousness of the damage. Thus, a basis would be available for determining more accurately the timing of spraying, particularly in connection with crop development and current weather. Sporulation of disease and its dissemination would most certainly come under more than one weather condition.

Summary

A physiological disease of onion leaves, called blast, is described.

The injury, in the nature of sunscald, results from the inability of the onion plant to adjust itself immediately to bright sunshine, high temperature, and low relative humidity, following a period of cloudy, wet weather concomitant with high temperature and high relative humidity.

Abnormal development of the plant in terms of soft tissue and a small root system during the subdued light period restricts the ability of the roots to replace the lost moisture which occurs when optimum conditions for transpiration appear. Tissue dehydration follows, especially in the areas exposed to direct sunlight.

The extent and seriousness of the injury is dependent upon the intensity of the two environments in relation to each other, and upon the stage of development of the crop.

Field observations have been supplemented with laboratory tests in which simulated weather conditions were employed. Sunscald, or blast, could be produced at will in the laboratory work.

A study of local weather records supplied data from which certain figures of cloudy weather, relative humidity, and hours of sunshine could be combined, indicating that blast would occur when this combination was changed to one of bright, sunny weather, high temperature, and low relative humidity.

The combinations of the first environment may be made visual by superimposing dials, each one of which can be adjusted according to the weather factor it represents.

The dial chart is described and explained.

The writer wishes to express his appreciation to Dr. W. G. COLBY for helpful suggestions.

MASSACHUSETTS STATE COLLEGE
AMHERST, MASSACHUSETTS

LITERATURE CITED

1. CLINTON, G. P. Report of the Station Botanist 1913. Connecticut Agr. Exp. Sta. Ann. Rept. 1914.
2. ————. Report of the Station Botanist 1915. Connecticut Agr. Exp. Sta. Ann. Rept. 1916.
3. DORAN, W. L., and BOURNE, A. I. Onion spraying and dusting experiments. Massachusetts Agr. Exp. Sta. Bull. 279. 1931.
4. METEOROLOGICAL OBSERVATIONS. Massachusetts Agr. Exp. Sta. Meteorological Series.
5. WHETZEL, H. H. Onion blight. New York (Cornell) Agr. Exp. Sta. Bull. 218. 1904.

SEMI-MICRO ESTIMATION OF REDUCING SUGARS

H. WEINMANN

Introduction

The estimation of sugars is a matter of interest to every worker engaged in the study of carbohydrate metabolism and related problems. In order to be of any real value a method must not only allow the determination of relatively small amounts of sugars with sufficient accuracy, but should also be rapid, and thus permit of a large number of determinations being carried out in a short time. Semi-micro modifications of the Munson-Walker-Bertrand method (**1, 5**) have been elaborated by ILJIN (**2**) and WILDMAN and HANSEN (**7**) with the object of satisfying these demands as nearly as possible. The method of WILDMAN and HANSEN unfortunately requires apparatus which is not available in every laboratory. ILJIN'S modification attracted the writer's attention on account of its simplicity.

In ILJIN'S method centrifuge tubes containing 6-ml. aliquots of the sugar solution and 6 ml. of Fehling's solution (of a higher concentration than used in the original Munson-Walker method) are heated in a boiling water-bath for ten minutes. After cooling, the precipitate is separated from the liquid by centrifuging, washed, and dissolved in acid ferric ammonium sulphate solution. The latter is then titrated with potassium permanganate solution.

Preliminary experimentation

When Iljin's method was tried out in the writer's laboratory consistently low values were obtained. It was first thought that these low values were due to the reduced boiling temperature of water (94° C.) at the altitude of Johannesburg (5,740 feet above sea level). The same low values were, however, obtained when the copper reduction was carried out in an oil-bath kept at 100° C. It was then decided to re-standardize the whole method, and at the same time to introduce a number of modifications which would render it simpler, more accurate and more rapid.

For all further experiments 5-ml. aliquots of sugar solutions and 5 ml. of Fehling's solution (composed of equal parts of A and B), of the same concentration as employed in the ordinary Munson-Walker method, were

TABLE I

EFFECT OF HEATING TIME ON COPPER REDUCTION BY GLUCOSE

MG. GLUCOSE PER 5-ML. ALIQUOT	MG. COPPER REDUCED BY HEATING FOR		
	3 MINUTES	5 MINUTES	10 MINUTES
	mg.	*mg.*	*mg.*
18.18	33.6	34.0	34.2
10.65	20.3	20.4	21.0
1.82	2.81	2.91	2.97

used. In order to find out whether the ten-minute period of heating in boiling water could be shortened, a series of pure glucose solutions were heated with Fehling's solution for periods of three, five, and ten minutes, respectively, and the amounts of reduced copper were determined (table I).

As can be seen from these figures little reduction took place after three minutes of heating, the reduced copper values for three minutes heating time being only 5 per cent. or less lower than those which were obtained with heating for ten minutes. Hence it appeared permissible to reduce the heating time from ten minutes to three minutes, particularly since a simple means was found of keeping to this time very accurately.

The modified procedure which was finally adopted as a result of further experiments is here outlined.

Solutions and apparatus required

Solutions

Fehling's A and B, equal proportions of which are mixed before use. Acid ferric ammonium sulphate solution. An (approximately) 0.05 N potassium permanganate solution, which is standardized against dry, purest sodium oxalate. All solutions are prepared as directed by Loomis and Shull (3).

Apparatus

Apart from the ordinary equipment available in every laboratory the following are required: 1. A centrifuge for use with 15-ml. taper-end tubes, and capable of making at least 2000 r.p.m. The writer used an Adams Senior electric centrifuge, holding six tubes, for all experiments. This was always run at full speed, *i.e.*, approximately 2700 r.p.m. 2. A small basket of wire mesh, large enough to hold one set of centrifuge tubes, and provided with a handle so that it can be suspended from a retort stand. Such a basket is easily made. This wire basket is used for immersing the centrifuge tubes containing the sugar-Fehling's mixture in the boiling water-bath. In this way, not only can all tubes of one set be handled simultaneously, but it is also possible to observe the heating time with the greatest accuracy.

Procedure

Pipette 5-ml. aliquots of the solution to be tested into 15-ml. centrifuge tubes. This volume of liquid must not contain more than 20 mg. of glucose or an amount of other reducing sugars of equivalent reducing power. If the solution contains more than 20 mg. of glucose in 5 ml. liquid, use smaller aliquots, and make up to 5 ml. with distilled water. Add 5 ml. of the mixed Fehling's solutions. Place the tubes in the wire-basket and immerse them in a boiling water-bath for *exactly* three minutes, attaching the wire-basket to the clamp of a retort stand. The volume of the water-bath and of the water must be sufficiently large for the water not to stop boiling when the tubes are immersed. The level of the liquid in the immersed tubes should

be slightly below the level of the boiling water. The water should only boil gently to prevent water splashing into the tubes.

At the end of three minutes place the wire basket holding the tubes into a beaker filled with running tap-water, and leave them there for three minutes. Then centrifuge for three minutes at 2000 to 3000 r.p.m. Using a small-bore glass tube, whose lower end is bent upwards and whose upper end is connected by means of a rubber tube to a suction flask and suction pump, carefully siphon off the supernatant liquid, leaving, however, approximately 0.5 ml. of liquid covering the precipitate in the tube. By gentle shaking and tapping disperse the bulk of the precipitate in the remaining 0.5 ml. liquid, and break it up as completely as possible by running in 12 ml. of distilled water in a strong jet from a burette. Any precipitate clinging to the walls should also be washed down by this means.

Centrifuge again for three minutes, siphon off, and wash as before. Centrifuge for a third time for three minutes, and again siphon off the supernatant liquid. After the precipitate has again been dispersed in the remaining 0.5 ml. liquid, vigorously blow in 5 ml. of acid ferric ammonium sulphate solution from a pipette, thus dissolving the precipitate.

If the whole precipitate does not dissolve immediately, as is the case particularly with larger precipitates, place the tubes for a few minutes in a beaker of hot water. Stir up any undissolved precipitate with a 5-ml. pipette. Suck up the liquid several times into the pipette, and allow it to run back again into the tube, at the same time rinsing the entire inner surface of the tube in order to dissolve any precipitate that may adhere to the glass. Wash the pipette into the tube with a little distilled water. Transfer the contents of the tube quantitatively into a small Erlenmeyer flask with a few washings of distilled water from a wash bottle, and titrate with the potassium permanganate solution to the faintest permanent pink.

For the estimation of amounts of glucose less than 1 mg. a 0.01 N permanganate solution should be used, titrating with a 5-ml. micro-burette. This dilute solution should not be stored long, but can be prepared rapidly before use by diluting the 0.05 N $KMnO_4$ solution. A 10-ml. micro-burette is also advantageously used for the determination of higher amounts of glucose (up to about 16 mg.) when titrating with the 0.05 N $KMnO_4$ solution. Duplicates will, in general, check within 0.05 ml. even with the more dilute permanganate solution. The most accurate and consistent results are obtained with amounts of glucose ranging from 1 to 10 mg. Amounts of glucose lower than 1 mg. can be accurately determined to the nearest 0.1 mg. only; such low concentrations should be used only if unavoidable or if no great accuracy is required.

Blank determinations (using 5 ml. of distilled water in place of the sugar solution) should be carried out from time to time. The blank values, which are subtracted from the titration values, will, however, not usually exceed 0.3 ml. of 0.01 N $KMnO_4$ solution or 0.06 ml. of 0.05 N $KMnO_4$ solution (equivalent to approximately 0.1 mg. glucose). For many practical pur-

poses, particularly if higher concentrations of reducing sugars are used, blank values may be neglected altogether.

From the quantity of reduced copper the amount of glucose is found by reference to table II. This table was computed from the results of a large number of determinations on solutions of pure, anhydrous glucose (B.D.H. Laboratory Reagent). The latter was stored in a vacuum desiccator over phosphoric oxide for several weeks before being used.

When using a centrifuge holding six tubes it is possible to complete six determinations in ¾ hour to one hour. With the help of an assistant a new

TABLE II

COPPER-GLUCOSE EQUIVALENTS, MODIFIED ILJIN METHOD

Mg. Cu	Mg. glucose	Mg. Cu	Mg. glucose
mg.	*mg.*	*mg.*	*mg.*
0.2	0.1	11.6	6.0
0.4	0.2	13.5	7.0
0.6	0.3	15.5	8.0
0.8	0.4	17.4	9.0
1.0	0.5	19.3	10.0
1.2	0.6	21.2	11.0
1.4	0.7	23.1	12.0
1.6	0.8	25.0	13.0
1.8	0.9	26.9	14.0
2.0	1.0	28.8	15.0
4.0	2.0	30.7	16.0
5.9	3.0	32.5	17.0
7.8	4.0	34.3	18.0
9.7	5.0	36.0	19.0
.....	.....	37.6	20.0

set can be started every ½ hour, and thus twelve determinations can be made in one to 1¼ hours.

Experimental data

TESTS WITH PURE SUGAR SOLUTIONS

In many phases of plant physiological research, where a separate estimation of individual sugars is not required, it is customary to calculate total reducing sugars as glucose. The question arises as to whether this procedure is justified, and particularly, whether different methods give comparable values. In order to investigate this question, solutions of eight of the most important reducing sugars, including the two reducing disaccharides, lactose and maltose, were tested both by the original Munson-Walker-Bertrand method and by the new modification. Glucose was included in this series as a check. Dry and pure preparations of these sugars were used, and in each case three different concentrations were tested, representing the lower, medium and higher part of the range. The reducing power of these solutions was then calculated as glucose from the copper-glucose curves of the method employed, and expressed as a percentage. The results are given in table III.

In most cases, the agreement in the glucose values as found by the two methods was very good, though with some sugars (fructose, galactose, and xylose) slightly lower values were obtained with the macro-method. The differences are, however, small enough to be considered to be of the mag-

TABLE III

REDUCING POWER OF PURE SUGAR SOLUTIONS AS DETERMINED BY THE MODIFIED ILJIN METHOD (A) AND BY THE MUNSON-WALKER-BERTRAND METHOD (B)

MONOSACCHARIDES

SUGAR	PERCENTAGE USED	PERCENTAGE FOUND AS GLUCOSE	
		A	B
	%	%	%
Glucose	0.330	0.324	0.326
	0.101	0.102	0.099
	0.0101	0.0101	0.0096
	0.304	0.279	0.267
Fructose	0.101	0.092	0.088
	0.0101	0.0102	0.0088
	0.317	0.290	0.286
Galactose	0.106	0.098	0.091
	0.0106	0.0102	0.0092
	0.186	0.168	0.172
Mannose	0.075	0.066	0.069
	0.0075	0.0066	0.0069
	0.297	0.300	0.303
Arabinose	0.099	0.101	0.098
	0.0099	0.0099	0.0094
	0.166	0.166	0.161
Xylose	0.066	0.067	0.063
	0.0066	0.0066	0.0063

DISACCHARIDES

SUGAR	PERCENTAGE USED	PERCENTAGE FOUND AS GLUCOSE		PERCENTAGE FOUND AS DISACCHARIDE	RATIO
		A	B	B	
	%	%	%	%	%
Maltose	0.521	0.272	0.275	0.497	1.92
$C_{12}H_{22}O_{11} + H_2O$	0.210	0.106	0.105	0.198	1.98
	0.0210	0.0106	0.0099	0.0160	1.98
Lactose	0.481	0.315	0.320	0.470	1.53
$C_{12}H_{22}O_{11} + H_2O$	0.193	0.125	0.122	0.187	1.54
	0.0193	0.0123	0.0120	0.0182	1.57

nitude of ordinary experimental error. It may be concluded that the two methods produced results which are well comparable for all practical purposes.

Comparing the actual sugar percentages used with the apparent glucose values found, good agreement was generally obtained for the monosaccharides with the semi-micro method, though somewhat low values were obtained for fructose, galactose, and mannose. The macro-method yielded

slightly low values for mannose and xylose, and still lower values than the new modification for fructose and galactose. Out of the 18 tests made on monosaccharide solutions, in 13 cases the glucose values found by the semi-micro method approach the sugar percentages used more closely than do the results obtained with the original Munson-Walker-Bertrand method. All deviations from the "expected" values are small, and it appears permissible from the practical point of view to calculate the sugar content of such solutions, or their mixtures, as glucose.

Entirely incorrect values are, however, obtained if lactose or maltose are calculated as glucose, on account of the lower reducing power of these disaccharides. Lactose is of little interest to the plant physiologist since it has not been detected in plants (**6**). Maltose, however, appears to occur widely distributed in plants, though generally in very small quantities. It is found in greatest amounts in germinating starchy seeds (**4**). When lactose and maltose were calculated as glucose, agreement between the two methods was found to be very good. The values obtained with the macro-method, and calculated as lactose and maltose respectively from the original Munson-Walker curves, are low, however.

The figures given in the column of table III, headed "ratio," represent the ratios of disaccharide concentration used: apparent glucose concentration found by the modified Iljin method. As will be seen, this ratio is more or less constant for different concentrations of the same disaccharide. The small differences found in the tests with different concentrations are considered to be due to experimental error. It is hence possible, when analyzing solutions which contain either lactose or maltose in the absence of other reducing sugars, to calculate the actual concentration of the disaccharide with sufficient accuracy by multiplying the apparent glucose value with the appropriate factor. The mean of the three values is proposed as a tentative factor, *viz.*, 1.96 for maltose, and 1.55 for lactose. Both factors refer to the crystallized disaccharide, containing one molecule of water of crystallization.

Tests with plant extracts

For a more practical test of the reliability of the new modification, extracts were prepared from a number of plant materials. Aliquots of the extracts were hydrolyzed, and unhydrolyzed and hydrolyzed aliquots of the extracts were used for the determination of reducing and total sugars respectively by both the macro- and the semi-micro method. Non-reducing sugars were found by subtraction (total sugars – reducing sugars) after the necessary corrections for preceding dilutions had been made. Both reducing and non-reducing sugars were calculated as glucose, and expressed as percentages of the analyzed extracts (not of the original materials). These values are given in table IV.

Here again good agreement was, in general, achieved in the results of the two methods. There are, however, several exceptions: the reducing sugar values of the extracts from bean leaves, grass roots, Eucalyptus

TABLE IV

SUGAR CONTENT OF VARIOUS PLANT EXTRACTS AS DETERMINED BY THE MODIFIED ILJIN METHOD (A) AND BY THE MUNSON-WALKER-BERTRAND METHOD (B)

MATERIAL	PERCENTAGE REDUCING SUGARS		PERCENTAGE NON-REDUCING SUGARS	
	A	B	A	B
	%	%	%	%
Grapes	0.190	0.188	0.005	0.005
Apple	0.151	0.149	0.042	0.041
Orange	0.065	0.063	0.083	0.082
Maize leaves	0.035	0.037	0.013	0.012
Bean leaves	0.006	0.021	0.021	0.025
Dried pasture herbage	0.020	0.028	0.065	0.066
Grass roots*	0.001	0.002	0.019	0.018
Grass roots;* starch hydrolysate	0.058	0.055	.....	.....
Grass roots;* polysaccharide hydrolysate	0.110	0.111	.....	.....
Eucalyptus manna	0.002	0.004	0.158	0.153

* Sample consisting of the roots of several indigenous South African grasses.

manna and (to a lesser extent) of the dried pasture herbage show relatively unsatisfactory agreement. Since the two methods gave comparable results with all the pure sugar solutions tested, it might be suspected that these discrepancies were due to the presence of other reducing substances, whose reducing power might vary under different conditions. On the other hand, it will be noticed that all discrepancies occurred with very low sugar concentrations.

In order to find out whether the accuracy of both methods is affected by the concentration of the extract used, a number of different dilutions were prepared from two of the extracts. The extracts used for this series were the grass root polysaccharide hydrolysate and the Eucalyptus manna extract. Of the latter material a new, more concentrated extract was pre-

TABLE V

EFFECT OF THE RELATIVE CONCENTRATIONS OF EXTRACTS ON THE ACCURACY OF THE MODIFIED ILJIN METHOD (A) AND OF THE MUNSON-WALKER-BERTRAND METHOD (B)

RELATIVE CONCENTRATION OF EXTRACT	PERCENTAGE REDUCING SUGARS FOUND			
	POLYSACCHARIDE HYDROLYSATE		EUCALYPTUS MANNA EXTRACT	
	A	B	A	B
1	0.110	0.111	0.096	0.102
1/2	0.113	0.111	0.095	0.102
1/5	0.114	0.110	0.095	0.101
1/10	0.117	0.106	0.088	0.086
1/50	0.073*	0.110*	0.054*	0.070*
1/100	0.050*	0.056*	.....	.....

* Values obtained by extrapolation of copper-glucose curves.

pared, while in the case of the polysaccharide hydrolysate the original extract (the reducing sugar values of which are given in table IV) was used. Aliquots of these extracts were diluted to twice, five times, ten times, fifty times, and one hundred times their original volume. The percentages of reducing sugars in these aliquots were determined by both methods; and in order to make all results comparable, the values were multiplied by the corresponding dilution factor (*viz.*, 1, 2, 5, 10, 50, and 100 respectively). The results are given in table V.

Practically identical results were obtained by both methods with all higher concentrations, down to 1/10 of the original concentration. Agreement was better for the polysaccharide hydrolysate than for the Eucalyptus manna extract, for which somewhat low values were also obtained with the relative concentration 1/10. The Eucalyptus manna extract was an uncleared aqueous solution, containing small amounts of colloidal matter. Inaccurate results and poor agreement were obtained with concentrations below the actual range of either method with both extracts.

Although the possibility cannot be rejected that with some extracts different values may be obtained by the two methods, it would appear that at least some of the above-mentioned discrepancies are merely due to errors resulting from the use of very low concentrations. By choosing a suitable concentration well within the required range it should be possible to obtain reliable results with most extracts, particularly if the latter are well cleared.

Summary

1. A new modification of ILJIN's semi-micro method for the determination of reducing sugars is described. The method uses 5-ml. aliquots of the unknown solution, and allows amounts of glucose ranging from 0.1 mg. to 20 mg. to be estimated accurately to the nearest 0.1 mg.

2. Experimental data are presented which show that for six monosaccharides the method gives values which are acceptable for practical purposes. Factors are given for the calculation of lactose and maltose.

3. Determinations of reducing and non-reducing sugars in a number of plant extracts by the new method yielded values which in most cases checked well with those obtained by the Munson-Walker-Bertrand method. Certain discrepancies in the results of the two methods were investigated.

UNIVERSITY OF THE WITWATERSRAND
JOHANNESBURG, SOUTH AFRICA

LITERATURE CITED

1. BERTRAND, G. Le dosage des sucres réducteurs. Bull. Soc. Chim. ser. 3, **35**: 1285–1299. 1906.
2. ILJIN, W. S. Bestimmung des Zuckers mittels Fehlingscher Lösung und Zentrifugierens. Biochem. Zeitschr. **193**: 322–325. 1928.
3. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. First edition. McGraw-Hill Book Company, Inc. New York and London. 1937.

4. MILLER, E. C. Plant physiology with reference to the green plant. Second edition. McGraw-Hill Book Company, Inc. New York and London. 1938.
5. MUNSON, L. S., and WALKER, P. H. The unification of reducing sugar methods. Jour. Amer. Chem. Soc. **28**: 663–686. 1906.
6. STEELE, C. C. An introduction to plant biochemistry. London, G. Bell and Sons, Ltd. 1934.
7. WILDMAN, S. G., and HANSEN, E. A semi-micro method for the determination of reducing sugars. Plant Physiol. **15**: 719–725. 1940.

USE OF ROOT PRESSURES IN DETERMINING INJURY TO ROOTS BY CULTIVATION[1]

O. A. Leonard

A rapid method is desirable for determining the injury to roots caused by cultivating plants in different ways and with different implements. No satisfactory procedure of studying injury to roots by cultivation has been developed. Cultivators form such irregular patterns, in cultivating soil, that direct determination of injury to roots is difficult.

The method to be described is comparative. Positive root pressures are used in the method. Manometers are used to measure the pressures at which water is forced into the xylem. If some roots are cut, the pressure will drop until the rate of entry of water is sufficient to maintain a certain pressure, and in addition, to maintain a certain rate of water flow from the cut roots into the soil. It is logical to suppose that the degree of injury to roots (amount of xylem cut) would affect both the rate at which water is lost from the cut roots, and the root pressures.

The writer is aware that the method is neither entirely satisfactory nor perfect. It is assumed, however, that the principles upon which the method rests are sound, and that the results can give a better insight into injuries to roots resulting from cultivation than has previously been possible.

Procedure

Cotton plants were detached with square cuts below the cotyledons, and short pieces of tight-fitting rubber tubing placed on the stumps, using rubber tubing expanders. Usually more plants were thus treated than were needed since some of the stumps failed to exude water. Capillary Pyrex, U-tube like manometers of a sufficient length to measure 45 cm. of mercury were attached to 10 exuding cotton stumps in each test.

After the manometers were attached, usually about 10 minutes were required for the root pressures to become sufficiently stable for use. The upper and lower portions of the mercury were rapidly marked, using a wax pencil. The root pruning instrument (cultivator or knife) directly followed the above operation. In approximately two minutes the top of the mercury column was again marked with a wax pencil and the change in height of the mercury measured with a centimeter rule. Readings were taken on similar but uncultivated plots to check on the natural variations in root pressures that occurred during the experimental period of two minutes.

[1] The preliminary experiments were conducted by Tilden Easley in 1938. The work was conducted in connection with a weed control and tillage project, supervised by T. N. Jones. Published with the approval of the Director, Mississippi Experiment Station, as paper no. 72. New series.

Results

Effect of time after cutting roots on root pressures

The data in table I were collected on the West Point, Mississippi, Experimental Farm. The soil type was Houston clay. The cotton was two months old, the plants 12 inches tall, the tap roots 12 inches deep, and the longest roots traced were 20 inches long. There were almost no roots below five inches; the water table was about 18 inches deep; and the soil oxygen values low, as judged by later studies on the same soil.

About 10 manometers were used in each test, and at the same time a similar number of manometers on undisturbed ground was used as checks. The soil had been cultivated about a week previous to the experiment, which greatly reduced the number of roots in the upper 2 inches of soil. The soil

TABLE I

Effect of time after a root pruning* experiment on changes in root pressure

Treatment	Number of plants tested	Percentage decrease of pressure					
		Change in pressure 2 min.	Change in pressure minus check	Change in pressure 7 min.	Change in pressure minus check	Change in pressure 17 min.	Change in pressure minus check
		%	%	%	%	%	%
Check	32	1	0	7	0	17	0
1″ deep × 6″ from row	10	3	2	6	−1	.....	.....
2″ deep × 6″ from row	10	3	2	7	0	.....	.....
4″ deep × 6″ from row	8	96	95	97	90	100	83
1″ deep × 12″ from row	9	4	3	12	5	20	3
2″ deep × 12″ from row	10	7	6	13	7	20	3
4″ deep × 12″ from row	10	43	42	54	47	59	42

* The soil was cut with a knife at various depths and distances from the cotton rows to obtain variable changes in pressure.

was cut with a heavy knife parallel to the cotton rows and at the indicated depths and distances from the plants.

Root pressure readings were taken after 2, 7, and 17 minutes. As can be seen in table I, the 2-minute period gave less time for natural variations in the root pressures to influence the results than did the 7- and 17-minute intervals. When compared with the check, almost the entire change in root pressure occurred within the first 2 minutes after the roots were cut. Apparently, 2 minutes was sufficient time for the root pressures to have approached sufficient stability for measuring changes in root pressure.

The data show that the root pressures were not appreciably affected, except by cutting the soil about 4 inches deep. The most marked reduction in

root pressures occurred by cutting the soil about 4 inches deep, 6 inches away from the plants. The data on changes in root pressures correspond with the observations on root distribution.

Relationship between percentage decrease in root pressures and percentage of roots cut

A few observations on the relationship between decrease in root pressure and injury to roots were made, using plants grown in nutrient solutions. These results indicated that both the number and size of the roots cut affected the percentage decrease in root pressure.

Some experiments were conducted in a cotton field to obtain more exact information on the relationship between the percentage decrease in root pressures and in the percentage of roots cut. The cotton tested was on a plot of uncultivated Houston clay soil with the weeds controlled by hoeing.

TABLE II

Relationship between the percentage decrease in root pressure* and the percentage distribution of roots (by weight)†

Depth	Decrease in root pressure (2 min.)	Fresh weight of roots in upper 5 inches
	%	%
1″	0	0
2″	15.8	15
3″	33.6	42
4″	50.0	66
5″	76.8	100

* Each reading is the average from 15 different plants.
† The percentage distribution of roots is the average from 15 different plants.

The plants were 2½ months old, 14 inches high, and had some roots as deep as 24 inches in the soil but with most of the roots less than 18 inches deep.

Manometers were attached to 75 different cotton stumps, 15 for each depth to be tested. The soil was cut vertically with a large knife, 6 inches from the plants, parallel with the cotton rows. The cuts were 1 foot long and 1, 2, 3, 4, and 5 inches deep. The manometers were read 2 minutes after each cut was made and were then disconnected. The root pressures, which were unusually high, varied from 40 to 60 cm. of mercury in the above test.

In order to compare the percentage decrease in root pressures with percentage of roots cut, rectangular samples of soil 6 to 10 inches from the rows and 1 foot long were taken at 0 to 1, 1 to 2, 2 to 3, and 4 to 5 inch depths; thus each sample contained the roots in 48 cubic inches of soil. The soil was washed through a fine screen, the roots picked off of the screen with forceps, washed, the excess water removed with filter paper, and weighed. Fifteen different plants were sampled and all of the weights kept separate, so that the variability between different plants could be determined. The results are shown in table II.

When the soil was cut 1 inch deep, there was no change in root pressure and there were no roots cut. A cut 2 inches deep resulted in a decrease in root pressure of 15.8 per cent. and in 15 per cent. of the roots cut in the upper 5 inches of soil. Soil cuts made 3, 4, and 5 inches deep resulted progressively in greater decreases in root pressure and progressively in greater percentages of roots cut.

A relationship is established between the quantity of roots cut and in the percentage decrease in root pressure. A given percentage decrease in root pressure appears to be about in direct proportion to the weight of roots cut. Considerable variability was noted between different plants in percentage changes in root pressure, although the root pruning operations were identical. These same differences between different plants were apparent in the weights and distribution of roots, as determined through soil sampling. Plants that appeared identical did not necessarily have their roots distributed laterally in the soil in exactly the same manner.

Recovery of root pressures after a cultivation

In a cultivation test, the root pressure averaged 24.7 cm. upon a plot that had never been cultivated, while upon a plot that had been cultivated 7 days previous to the test, the pressure averaged 25.1 cm., or essentially the same values. Directly after being cultivated, the pressure on the cultivated plot was only 34 per cent. of the uncultivated plot; 24 hours later it was 59 per cent. of the uncultivated plot. Almost identical results were obtained in another test.

These data indicate that root pressures recover rapidly after a cultivation. Complete recovery in pressures had occurred in 7 days.

Cultivation tests

These tests were conducted on Sarpy sandy loam soil at the Delta Branch Experiment Station, Stoneville, Mississippi.

Table III shows the results of a cultivation test that was made after a rainy period of three weeks during which time it was not possible to cultivate. The plants were about 2 feet high, tap roots 24 inches deep, and lateral roots about 30 inches long. The fresh weights of roots from the uncultivated plot, taken at 2-inch intervals, showed a fairly uniform distribution with depth. In general, the subsoil had as high a base exchange capacity, and was just as fertile, as the surface soil. The soil oxygen percentages were close to 20 per cent. at all depths studied from 8 inches to 6 feet. The above factors probably account for the fairly uniform distribution of cotton roots.

The cultivation tests were: (1) no cultivation; (2) deep cultivation (6″); (3) shallow cultivation (3″); (4) disc cultivation (2″); and (5) harrow cultivation (1″). The depths of cultivation are estimates since the exact depths were difficult to determine. The data show that the more deeply cotton was cultivated, the greater was the reduction in root pres-

TABLE III

EFFECT OF DIFFERENT METHODS OF CULTIVATING COTTON* UPON ROOT PRESSURES. SARPY SANDY LOAM

METHOD OF CULTIVATION	PERCENTAGE DECREASE OF ROOT PRESSURE IN 2 MINUTES
	%
Check	1
Cultivate deep (6″) tractor	72
Cultivate shallow (3″) tractor	53
Disc (2″)	32
Harrow (1″) spike tooth	26

* The cotton had not been cultivated for three weeks because of wet weather.

sure. Since the cotton had been cultivated 3 weeks previously by the same implements and at the same depths, the roots injured were less than 3 weeks old.

Table IV shows the results of another test conducted on the same soil as the one in table III, but after a dry period when it was possible to cultivate each week. The plants were about 15 inches tall, the tap roots about 22 inches deep, and some laterals about 20 inches long. The top soil was moderately dry. The results show that deep cultivation resulted in no more injury than harrowing, the shallowest method used. The fact that the cultivations were one week apart apparently did not allow sufficient time for

TABLE IV

THE EFFECT OF DIFFERENT METHODS OF CULTIVATION ON THE PERCENTAGE DECREASE IN ROOT PRESSURES OF INDIVIDUAL PLANTS AND AVERAGES PER TEST. SARPY SANDY LOAM

PLANT NO.	DECREASE OF ROOT PRESSURE IN 2 MINUTES					
	NO CULTIVATION	HARROW (SPIKE TOOTH) (1″)	DISC (2″)	TRACTOR CULTIVATE SHALLOW (3″)	TRACTOR CULTIVATE DEEP (6″)	CULTIVATE DEEP (6″) PREVIOUS CULTIVATIONS SHALLOW (3″)
	%	%	%	%	%	%
1	0.0	1.0	23.0	23.8	0.0	54.2
2	0.0	1.6	0.0	0.0	19.3	92.3
3	0.0	0.0	6.2	15.5	18.9	16.6
4	3.6	11.3	9.4	20.3	14.2	72.7
5	0.5	1.9	18.0	20.6	0.0	50.0
6	4.0	11.0	33.3	10.3	14.0	100.0
7	1.8	14.0	7.3	0.0	0.0	100.0
8	0.0	4.4	18.9	15.5	6.8	43.7
9	2.0	2.0	37.7	0.0	0.0	64.0
10	1.3	3.7	27.5	0.0	0.0	100.0
11	+ 3.0	5.8	12.0	.....	.....	.....
12	0.0	14.0	21.0	.....	.....	.....
13	0.0	0.0	.....	.....	.....	.....
14	1.7	4.6	.....	.....	.....	.....
15	1.7	3.5	.....	.....	.....	.....
Average decrease in pressure	0.9	5.2	17.8	11.1	7.3	69.3

roots to have penetrated very far into the previously cultivated soil; another factor influencing these results was the moderately dry nature of the surface soil. Readings from individual plants are given to indicate the variations that should be expected. These variations were due to differences in distribution of the cotton roots. The fact that cultivation did not injure all plants to the same degree is evident from the results. The differences in results between plants indicate that a variable number of large roots were cut. The small roots fairly uniformly filled the soil.

A plot which had previously been cultivated shallow was cultivated deep; this treatment resulted in a decrease in root pressure of 69.3 per cent. The decrease in root pressure by cultivating deep on a plot which had previously been cultivated deep was 7.3 per cent. It is obvious that many more roots were cut in the former than in the latter case.

Discussion

This method of measuring injury to roots while cultivating is dependent upon positive root pressures; therefore, it is best to test the plants before planning an experiment. The soil must be moist. According to KRAMER (**2**) the soil should have a moisture content of 45 per cent. or more between the moisture equivalent and the wilting percentage in order to exude water.

Before cotton is cultivated, the manometers measure the internal pressure in the closed xylem system. The pressure at equilibrium [in reality there is no true equilibrium, GROSSENBACHER (**1**)] is such as to prevent any further entrance of water into the xylem. When roots are cut the xylem becomes open and water flows from the xylem into the soil. As the pressure drops, water again flows into the xylem from all of the uncut roots. The decrease in root pressure would seem to be related to the rate of entry of water into the roots by absorption as balanced against the rate of exit of water from cut roots by bleeding. The data in table I indicate that 2 minutes gave sufficient time for the balance between the rates of entry and exit of water to occur. Since root pressures are never stationary, it is necessary to read the manometers, after a root pruning experiment, as soon as the pressures approach stability.

The decrease in root pressure is somewhat proportional to the quantity of the xylem cut as was shown in table II. The exact relationship is complicated and difficult to determine.

The maximum reduction in root pressure occurs directly after cultivation. After one day the reduction in root pressure is considerably less, and after one week there is no reduction in root pressures when compared with uncultivated cotton. It appears as though a week is sufficient to stop the loss of water from cut roots and, therefore, is sufficient time between experiments.

The following precautions should be observed: (1) similar sized plants should be used; (2) the manometers should have small, similar sized capillaries; (3) the manometers should have a length sufficient to measure 45 cm.

of mercury; (4) the manometers should fit tightly against the tops of the plant stumps, and the rubber attaching the manometer to the stump should be fairly rigid; (5) plants should not be tested unless a week or more has elapsed since the previous cultivation; and (6) the plants should be tested for positive pressures before starting an experiment.

Summary

A method of measuring injury (comparatively) to roots by cultivation has been described. The method involves using changes in positive root pressures before and after cultivation, with a standardized time interval of two minutes between readings. The results are calculated as percentage changes in root pressure.

MISSISSIPPI EXPERIMENT STATION
STATE COLLEGE, MISSISSIPPI

LITERATURE CITED

1. GROSSENBACHER, K. A. Diurnal fluctuation in root pressure. Plant Physiol. **13**: 669–676. 1938.
2. KRAMER, P. J. Soil moisture as a limiting factor for active absorption and root pressure. Amer. Jour. Bot. **28**: 448–451. 1941.

BUFFER VALUES OF THE LEAVES OF SOME PLANTS

GEORGE E. F. BREWER AND EDITH GODAR

(WITH TWO FIGURES)

Introduction

One of the factors which influence the chemical reactions within the tissue of a living plant is the actual hydrogen ion concentration. There are at least two external factors which might affect the acidity of the living cell: (1) the CO_2 content of the air; and (2) the acidity or alkalinity of the soil. It seems important, therefore, to know what resistance a plant can offer against a change of its actual acidity. If, for instance, base or acid is added to distilled water, a comparatively big change of pH is observed (curve 1, fig. 1), whereas a solution of 4 gm. NaOH and 12 gm. acetic acid (per liter of distilled water) shows only a slight change of pH upon addition of base or acid (curve 3, fig. 1). The term buffer index has been introduced to describe these circumstances. The mathematical argument for the buffer index "β" was developed by VAN SLYKE (**9**). It is the differential quotient of the gram equivalent concentration of base or acid added, with respect to the change in pH, $\frac{dB}{dpH}$.

$$\beta = \frac{\text{Normality of base (or acid) added} \times \text{ml. base (or acid) added}}{\text{Shift in pH} \times \text{volume (or weight) of buffer solution}}.$$

A considerable amount of work has been done to determine the titration curves and buffer index curves of plant tissues, using expressed sap, filtered or unfiltered, diluted or undiluted, boiled or unboiled. In this work we have determined the pH, titration curves, and buffer indices of some plant material which have not been determined hitherto, and have also, for comparison, included some plant material about which data have been reported previously, extending these data over a wider range.

Method

The method used consists in macerating 0.5000 gm. of plant tissue with a small quantity of water, and diluting the resulting suspension to 5 ml. This suspension is immediately put into the cup of a glass electrode set, and the initial pH determined. Small quantities of acid or base are added to the suspension of macerated plant tissue, using a microburette calibrated to 0.01 ml., which allows the estimation of 0.001 ml. After each addition of acid or base, and a few seconds of stirring, the pH is recorded. In all cases investigated, the pH remained constant for a period of at least 15 to 20 minutes, so that we may safely conclude that equilibrium had been reached. The data so recorded include weight of tissue, volume of solution, the number of ml. of acid or base added, the normality of the acid or base

added, and the shift in pH. These data were used to plot acidity against the volume of the acid or base (fig. 1), which diagrams are usually called the titration curves. From these the buffer indices were computed and then plotted against pH (buffer index curves, fig. 2).

It should be noted that we based our computation on the weight of plant

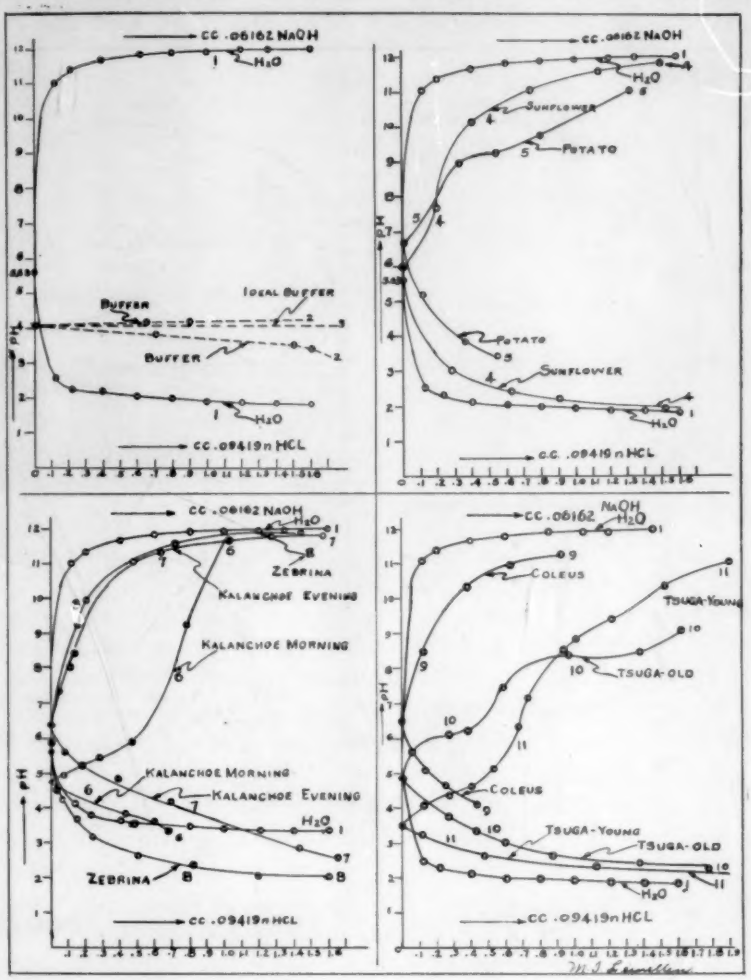


FIG. 1. Titration curves.

material used and not on the weight of solution in order to simplify the comparison with the data given by other authors.

Regarding the influence of dilution upon pH, we quote MARTIN (5). ". . . Since dilution does not change the ratio of acid salt to normal salt in dilute concentration, the pH value of the sap on dilution should not change to any appreciable extent. . . ." This was proved by her experiments, and our results also confirm the statement.

Results

SUNFLOWER (*Helianthus annuus*).—Sunflower seeds were soaked for 24 hours in water and then grown in garden soil, indoors, near a south exposed window in April. The cotyledons for the experiment were picked before the first leaves unfolded (see curves 4, fig. 1; and 13, fig. 2).

POTATO (*Solanum tuberosum*).—A tuber which had been stored over

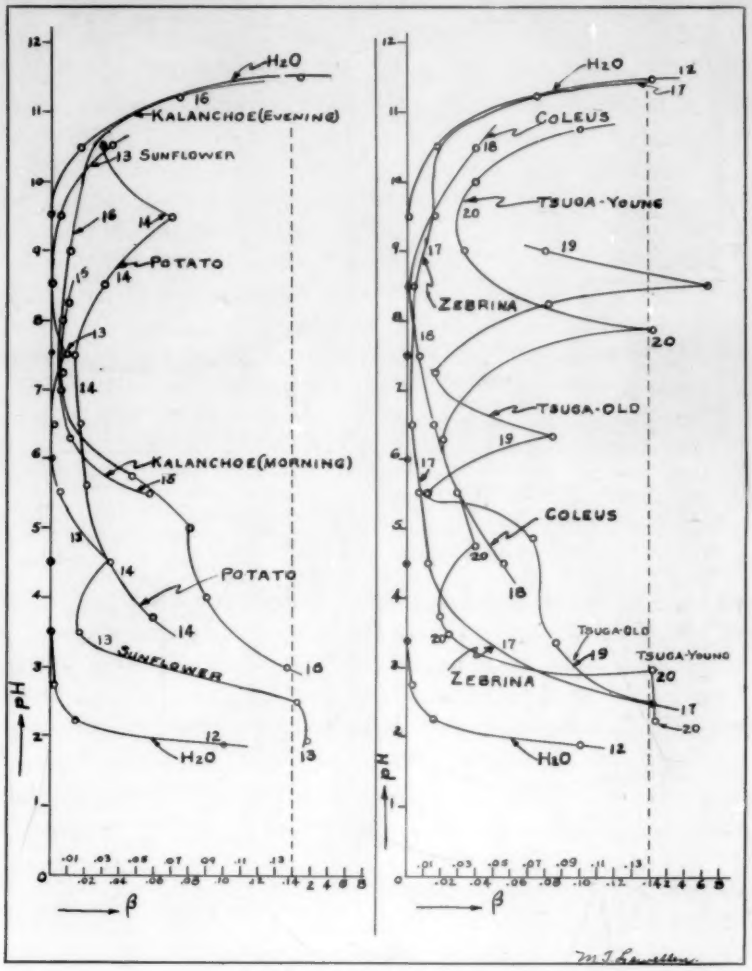


FIG. 2. Buffer index curves.

winter was cut in half and placed cut side downward in a glass jar, after a few ml. of water had been added. The experiment was carried out using the inner storage tissue, when the new roots in the older eyes were about two inches long (see curves 5, fig. 1; and 14, fig. 2).

BRYOPHYLLUM (*Kalanchoe pinnata*).—The experiments were carried out on the lowest leaves of a plant which carried about 10 pairs of leaves

(about 10 inches high), grown indoors in sandy soil. After a succession of rainy days, the plant was placed in darkness and the "morning" experiperiment was carried out the next morning, when the plant had been in darkness for 16 hours (see curves 6, fig. 1; and 15, fig. 2). The "evening" experiment was carried out a few days later, after the plant had been exposed to sunlight for 16 hours (see curves 7, fig. 1; and 16, fig. 2).

WANDERING JEW (*Zebrina pendula*).—The leaves for this experiment were selected from a branch about 10 inches long, which was grown indoors in garden soil. Care was taken to select pieces which contained the normal proportion of green, purple, and silvery tissue (see curves 8, fig. 1; and 17, fig. 2).

COLEUS (*C. blumei*).—The experiment was carried out using the leaves (1 inch in diameter) of a plant about 5 inches high, grown indoors in garden soil. The leaves were variegated, green, white, and red; and the experimental material was selected to show about the same average composition in green, white, and red as a normal leaf (see curves 9, fig. 1; and 18, fig. 2).

DOUGLAS FIR (*Pseudotsuga taxifolia*).—The leaves for the experiment were selected from a tree about 8 ft. tall, 10 years old. It had grown outdoors in garden soil. For one experiment, the soft green young needles were picked; for the other experiment, mature needles which grew next to the young ones were chosen (see curves 10 and 11, fig. 1; and 19 and 20, fig. 2).

Discussion

SUNFLOWER (*Helianthus annuus*).—Miss MARTIN (**6**) reports for the hypocotyl a VAN SLYKE index β equal to 0.0020, which compares well with our determination of β equal to 0.0020 for the same pH range (pH 5 to 7).

POTATO (*Solanum tuberosum*).—Our data compare well with the data given by COHN, GROSS and JOHNSON (**2**) from which SMALL [(**8**) page 286] has computed the buffer indices, as well as with the data given by INGOLD (**4**).

BRYOPHYLLUM (*Kalanchoe pinnata*).—In the narrow range observed by INGOLD (**4**) our data are in agreement with his, especially if we consider the large diurnal fluctuations of acidity in this plant.

It is furthermore of interest that the titration curve of this plant, in its acid "morning" condition, finally approaches the H_2O-base curve sooner than the more alkaline "evening" titration curve does. This is in agreement with the general observation that a greater total acidity (titrable acidity) in plant tissue is concomitant with lesser actual acidity [see GUSTAFSON (**3**)]. This applies also to curves 10 and 11, figure 1.

COLEUS (*C. blumei*).—Our data show that the leaves of this plant are more highly buffered toward acid than toward alkali.

WANDERING JEW (*Zebrina pendula*).—Our data show that this plant is very slightly buffered against both alkali and acids.

DOUGLAS FIR (*Pseudotsuga taxifolia*).—The observed data show unsteady slopes of the titration and buffer curves, which seems to be characteristic for

the family of conifers, since CHAPMAN's (1) data point the same direction for the roots of *Pinus echinata* seedlings. The unsteady slope of the curves can be explained by the presence of polyvalent anions, which, like $PO_4^{\equiv}$ ion, give unsteady titration curves, or by the presence of several different anions of distinctly different ionization constants.

Summary

1. The pH values of several plant tissues were determined by the use of a comparatively speedy method and changes in the pH upon addition of small quantities of acid or base determined. The recorded data were plotted and the titration curves drawn (fig. 1).

2. From the titration curves the buffer indices "β" (VAN SLYKE indices) were computed. The VAN SLYKE indices "β" were plotted against pH and the buffer index curves drawn (fig. 2).

3. The data observed regarding sunflower, potato tuber, and *Kalanchoe pinnata* are in fair agreement with the data reported by other authors, using other methods, and data extending over a wider range of pH are hereby reported.

4. The data on *Coleus blumei* show that the leaves of this plant are comparatively well buffered against acids and only slightly buffered against alkali.

5. The data for *Zebrina pendula* show that the leaves of this plant are very slightly buffered against both acid and alkali.

6. The data on *Pseudotsuga taxifolia* show that the leaves of this plant contain polyvalent anions and/or several anions of distinctly different ionization constants.

ROSARY COLLEGE
RIVER FOREST, ILLINOIS

LITERATURE CITED

1. CHAPMAN, A. G. Tolerance of shortleaf pine seedlings for some variations in soluble calcium and H-ion concentration. Plant Physiol. **16**: 313–326. 1941.
2. COHN, E. J., GROSS, J., and JOHNSON, O. C. The isoelectric points of the proteins in certain vegetable juices. Jour. Gen. Physiol. **2**: 145–160. 1919.
3. GUSTAFSON, F. G. Total acidity compared with actual acidity of plant juices. Amer. Jour. Bot. **11**: 365–569. 1924.
4. INGOLD, C. T. The H-ion concentration of plant tissues. X. Buffers of the potato tuber. Protoplasma **3**: 458–469. 1929.
5. MARTIN, S. H. The H-ion concentration of plant tissues. III. The tissues of *Helianthus annuus*. Protoplasma **1**: 497–521. 1926.
6. ————————. IV. The buffer of sunflower hypocotyl. Protoplasma **1**: 522–536. 1927.

7. ————————. VII. The buffers of sunflower stem and root. Protoplasma **3**: 273–281. 1928.

8. SMALL, J. H-ion concentration in plant cells and tissues. Protoplasma Monograph. Borntraeger, Berlin. 1929.

9. VAN SLYKE, D. D. On the measurement of buffer values and on the relationship of buffer values to the dissociation constant to the buffer and the concentration and reaction of the buffer solution. Jour. Biol. Chem. **52**: 525–570. 1929.

BRIEF PAPERS

GLASS POTOMETERS FOR STUDIES OF ABSORPTION AND EXUDATION BY EXCISED ROOTS

H. F. Rosene

(WITH ONE FIGURE)

Two types of potometers which have been found very useful in studies on roots are shown in figure 1. Type I is used for studies of exudation only, type II for absorption and exudation.

The dimensions of a potometer of either type are based upon such factors as: the diameter and length of the root selected for study; capacity of the vessel, A, which should be sufficient to provide an adequate oxygen supply when gases are not passed through the solution surrounding the root; and by the type of apparatus in which the potometer is placed.

Each potometer is filled or drained through the tube, N, by using a small pipette with a rubber bulb and a short piece of rubber tubing to fit over the external orifice of N. This pipette is used to stir the solution when desired. In type I, tube N is also used to pass gases or solutions continuously into the vessel and out through the side arm, P. The external orifices of N and P are closed with minute glass stoppers or by clamping rubber tubing passed over the projecting side arms.

A root with no lateral branches is threaded through the respective holes, R, S, and T of each potometer. In type I the hole, S, extends through the glass cover (which in some potometers is attached with vaseline to permit easy removal); in type II, S is the external aperture of the neck. The diameters of R and S correspond in general to the diameters of the selected roots; they are a fraction greater to permit threading without injury.

Threading a root through the aperture is conveniently done by holding the plant and excising the root at the upper level of R after threading. Excision is always made with a sharp razor blade through a drop of water surrounding the root at R. The excised root is held in place by the walls of the hole, T, bored through a small rod attached to the vessel wall.

The root is placed so that its cut surface is just below the top edge of the opening R. A small drop of water over the cut edge provides a meniscus as a reference point at the beginning of an experiment. Exudate flows from the cut surface into the "exudation" tube, E, attached to the bent glass rod, B, which is the potometer support. When desirable the opening R is covered with a minute piece of cover glass.

An "absorption" tube, D, attached to the neck of a potometer vessel of type II is the essential difference between the two potometer types. This is filled or drained by passing fluid through tube N.

Determination of the rates of absorption or exudation is based upon observation of the excursions of the terminal menisci in the "absorption" or

"exudation" tubes. The diameter of the bore of these tubes, as used in studies on young onion roots, is usually 1 mm. or less, and a horizontal microscope fitted with a filar micrometer eye piece is used to make readings. If growth occurs in excised roots it is necessary to make corrections for the excursions of the meniscus in the "absorption" tube D (type II).

Potometers of both types are used for studies of straight roots without laterals; potometers of type II are also used to study exudation of excised root systems. The glass cover is removed to permit ready insertion of the root system into the vessel A; it is replaced by fitting it over the cut end of

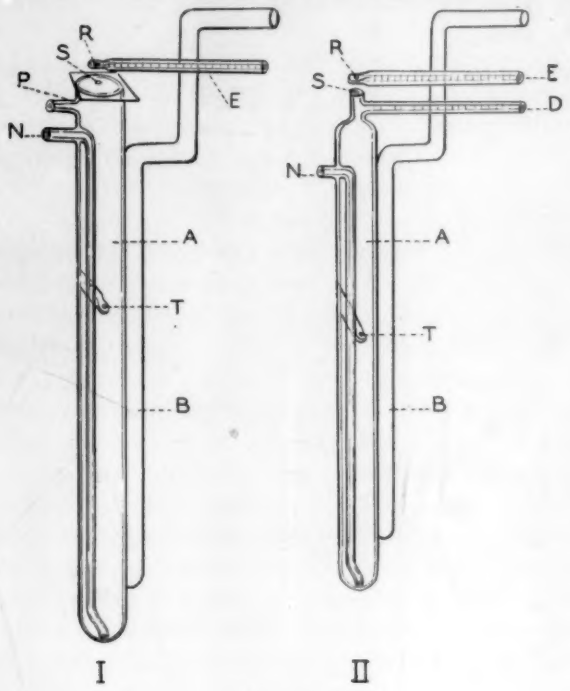


FIG. 1.

the main root. The cut end is inserted into R from below; the exudation tube E of type I is not placed in as close proximity to S as in the illustration thus permitting easy insertion from below.

In the studies on onion roots, potometers are placed within individual doubled walled metal chambers, rectangular in shape, and are supported by fastening the bent arm of B in a clamp attached to a chamber wall. The cover of each chamber is firmly held in place against a thick gasket of soft rubber. Two glass windows in the front and back walls permit light to enter and provide for observations. Two holes in opposite side walls of each chamber, face each other and are in line with the long axis of the exudation tubes and arm extensions of each potometer. These holes provide access into the closed chamber without removing the cover. Pipettes are

conveniently passed through them to fill or drain the vessels and tubes. The holes are closed with rubber stoppers. These openings in the side walls also provide for making connections to the external orifices of N and P (type I) for continuous flow of gases or solutions.

The lower portion of each chamber is flooded with water to a level just below the arm of tube N. This serves as a water bath and provides a humid atmosphere in the upper portion of the enclosed space.

Further temperature control is attained by circulating water through the space between the walls of each chamber.

UNIVERSITY OF TEXAS
AUSTIN, TEXAS

NOTES

Executive Committee Meeting.—The Executive Committee of the American Society of Plant Physiologists convened at the call of its officers in Chicago on January 29 and 30, 1944. The meetings were held in the Stevens Hotel, and eleven members were present. Many problems came under review, some of which could be settled immediately, while others depend upon future ratification, or upon the annual election. As rapidly as these matters are definitely decided, information will be provided for the membership.

The problems considered were mainly the following: Consideration of the report of the Committee on the Editorship; policies regarding future meetings during the war emergency; the problem of war emergency committees; closer cooperation between biological societies; plant physiological monographs; the Kilgore Bill; permanence for the offices, particularly those concerned with our permanent records and our financial assets; the strengthening of ties between our organization and the plant physiologists of Western Hemisphere republics; cooperation with the Scientific Monthly in publicizing the advances being made in plant physiology; routine matters, and appointments to committees.

An editorial board was provided for the monograph series, five members to serve for five years each, but to be started with one-, two-, three-, four-, and five-year terms, and one to be reappointed or replaced each year. The editors of the monograph series will be appointed after passage of the necessary legislation. Dr. LOOMIS reported that only a few copies of the first monograph are left. He has the outline for a monograph on photosynthesis, but actual publication will have to wait until the close of the war.

The Executive Committee voted unanimously that the Society become affiliated with the Union of American Biological Societies, with the distinct understanding that there is no financial responsibility connected with such affiliation.

The constitutional amendments recommended are too numerous to list, but most of them are related to changes in the names and functions of the officers heretofore called secretary-treasurer and business manager. If the changes are adopted, the secretary-treasurer will become secretary, and the business manager will become executive secretary-treasurer. The latter will handle all moneys, dues, and subscriptions, sales of back volumes, etc., pay all bills, and become an appointive instead of an elective officer. All of these important changes will appear upon the ballot at the next annual election in May.

Dr. BERNARD S. MEYER, president of the Society at present, was appointed to be Contributing Editor to Scientific Monthly, and he was also unanimously chosen to membership on the editorial board of Plant Physiology for a five-year term beginning in 1943.

Dr. WALTER THOMAS of the Pennsylvania State College was reappointed to membership on the finance committee. In connection with the financial position of the Society, the reserves, to the extent of $1,000.00, were ordered to be invested in a government bond, series G.

Closer bonds are sought between ourselves and plant physiologists of the South and Central American republics, and Mexico. Possibly members already residing in the other Americas may become ambassadors of good will to foster closer scientific and social ties among us. It is desirable and to our mutual advantage to have these ties of friendship as close and warm as possible.

The most important matter under consideration was the choice of an editor-in-chief to follow the present incumbent of that office. After a trial ballot which considered four names, the Executive Committee expressed its unanimous choice of Dr. WALTER F. LOEHWING of the State University of Iowa for this position. As the current editor-in-chief has not resigned, but merely wishes to let his natural appointive term expire, it was felt that the choice should be ratified by the Executive Committee as constituted after the first of July, since this appointment would naturally have been made at the December, 1944, meeting, were meetings being held. Some such procedure seemed desirable so that there might be some overlapping of time for consultation between the editors; the October number of PLANT PHYSIOLOGY should be ready for the printer by about July 1. Yet the Executive Committee desired not to seem in any way to exceed its authority, and provided for ratification of its action by the officers in charge after July 1, 1944.

Life Membership Awards.—The Charles Reid Barnes life membership committee has made two awards. The twentieth award, according to the by-laws, was to be made to a distinguished foreign plant physiologist. In accordance with this regulation, the committee has chosen Dr. N. G. CHOLODNY, professor of Plant Physiology at the University of Kiev, as the recipient of this honor, which is conferred upon scientists of other lands at each fifth award.

The committee also made the twenty-first award of the Charles Reid Barnes life membership to Dr. WALTER THOMAS of the Pennsylvania State College. Dr. THOMAS is well known for his studies of carbohydrate and nitrogen metabolism, plant nutrition, and in recent years for his studies of foliar diagnosis, the principles of which are becoming of greater importance as they are better understood and applied.

The American Society of Plant Physiologists is highly honored by these selections. There are 17 living members in this group.

Stephen Hales Award.—The ninth of the Stephen Hales awards will be due in December, 1944. The committee consists of the last three recipients of the award, the earliest of the three being chairman of the committee. Dr. JOHN W. SHIVE of the New Jersey Agricultural Experiment Station will,

therefore, be chairman; acting with him will be Dr. PHILIP R. WHITE of the Rockefeller Institute of Medical Research, Princeton, New Jersey, and Dr. CORNELIS BERNARDUS VAN NIEL of Stanford University. In recent years an attempt has been made to alternate the awards to older and younger men, but each committee has determined for itself what line of demarcation to make between younger and older. The award for 1944 should go to a younger man who has made outstanding contributions to plant physiology.

War Service.—Plant physiologists will be interested in the description of their profession which is now being used by the Bureau of Placement of the War Manpower Commission for men now on the National Roster of Scientific and Specialized Personnel. Publication of this material has been authorized by Leonard Carmichael, Director of the National Roster.

"Plant physiology: The basic science upon which rests many of the practical agricultural applications carried out in agronomy, ecology, floriculture, horticulture, pathology, and other plant sciences. It deals directly with the influence of each environmental condition, such as temperature, humidity, light, mineral nutrients, etc., on the individual processes going on in the plant, such as growth, respiration, photosynthesis, metabolism, nutrition, etc. Improvements in plant growth, propagation, and yields are direct applications of basic physiological studies of hormones, vitamins, and mineral nutrients. Modern storage and transportation of plant products are based on principles of respiration and metabolism. Important medical practices are frequently outgrowths of plant physiological studies."

Specialization in plant physiology and pathology is stated in the following terms: "Specialization in both these fields is usually by particular crop or group of related crops, although there is some specialization in the other dimension, for example, in the field of growth hormones, in plant nematology or in plant virology, in groups of diseases such as smuts and rusts, or in control measures, such as spraying or seed treatment."

The crop groups are listed as follows:

1. Forest trees, including log and shade trees.
2. Fruits and nuts, including grapes and berries.
3. Vegetable crops.
4. Flowers and ornamentals, including shrubs.
5. Cereal and grain crops.
6. Forage crops and grasses.
7. Field crops, including fiber plants, tobacco, potatoes.
8. Nursery and greenhouse crops.
9. Rubber, oil, or drug plants.

Functional specialization is described in terms of the nature of the work performed, the following in addition to any others already named:

1. Research.
2. Inspection (applicable to pathologists).
3. Extension (" " ").

4. Teaching at the university level.
5. Administration and management.

"In determining the professional status of an individual, some weight can be given to membership in a national or local professional organization. The top grade of membership in such groups is generally limited to individuals who have acceptable professional qualifications. Lack of such membership, however, should not be construed as indicative of nonprofessional status."

"A technically qualified plant pathologist or plant physiologist usually possesses the minimum of a bachelor's degree in either field, or occasionally in some related field as horticulture, agronomy, or botany."

"Plant physiology, although a botanical subject, is related to bio-chemistry, physics and physical chemistry."

"Plant pathologists and physiologists are employed principally by government, in higher education, in non-profit research agencies, and in food products and agricultural fungicides manufacturing industries."

These statements of the governmental authorities constitute valuable information to the plant physiologists whose status has not been determined in the war effort. Many are already engaged in definite war services, and the work of the plant physiologist has many vitally important angles, both for war, and for the reorganization of world peace.

Erratum.—The editor's attention has been called to an error in the October, 1943 number of PLANT PHYSIOLOGY. On page 629, table IX, first footnote to the table, for "ration" read ratio. Once more we call attention to the desirability of reports from all authors who find any errors in their papers. In spite of our efforts to keep errors out, they slip by occasionally; and we welcome the interest and watchfulness which leads to the reporting of mistakes. We urge authors to be meticulous in proofreading the galleys; if errors are noted at that time, there is better assurance that the papers will be letter perfect. Please correct the errors in your number of the journal.

Plants and Vitamins.—In recent years it has become obvious that vitamins are useful not only in animal metabolism, but that they are also important to the plants which produce them, and particularly to plants which do not produce enough of them for their own growth. An excellent book on this subject is *Plants and Vitamins* by W. H. SCHOPFER, Director of the Botanical Institute, The University of Bern. An authorized English translation has been provided by NORBERT L. NOECKER of Notre Dame, and the foreword is by W. J. ROBBINS.

There are twenty-four chapters, which are divided into three general sections or parts. Part 1 discusses the synthesis of vitamins in plants; auxo-autotrophic plants; and research methods. Nine of the chapters are included in part 1. There is a general introduction, followed by a discussion of the plant cell and its capacity for synthesis. Then come chapters on

experimental study of growth factors and the selection of test plants; classification and definition of active substances; the principal vitamins synthesized by plants; vitamins and their action on plants synthesizing them (two chapters); and the biosynthesis of vitamins (two chapters).

Part 2 contains eleven chapters, dealing with vitamins in relation to plants unable to synthesize them, and the growth factors of microorganisms. Thiamin and its components; yeast and bios; nicotinic acid and its amide; growth factors of the lactic bacteria; nitrogen-fixing bacteria and their growth factors, and coenzyme R (biotin); requirements of the hemophilic organisms, factors X and V; and such factors as ascorbic acid, vitamin D, cholesterol, pimelic acid, and the SH group, receive detailed consideration. The functions of the vitamins, especially as coenzymes; the capacity the organisms possess for synthesis, the effects of loss of synthetic capacity on the organism; and vitamins in relation to other active substances round out the survey.

The last section, part 3, deals with general problems involving vitamins. The four chapters are titled as follows: Vitamins in nature, their rôle in agriculture and horticulture, vitamin cycles; growth factors, vitamins, and sexuality; symbiosis, parasitism, and vitamins; and microorganisms as test objects for vitamins. Less than three pages set forth the conclusions, giving the gist of the matter as it were in a nut shell. An author index lists well toward six hundred authors, and an adequate subject index closes the work.

Here we have an excellent survey of the most recent developments in the field of plant nutrition, the functions of the trace organic essential nutrients. SCHOPFER is a leader in the development of the field, and the translator has rendered it into very good English. It is one of those books the investigator cannot afford to pass by. The publishers may be addressed Waltham, Massachusetts, the Chronica Botanica Co.; New York City, G. E. Stechert and Co. The book is priced at $4.75 per copy.

Statistical Analysis.—The importance of statistical analysis has been steadily growing, and we call attention to the recent publication of *Statistical Analysis in Biology*, by K. MATHER, head of the Genetics Department of the John Innes Horticultural Institution, London. The book differs somewhat in its aims from other excellent books on the subject, by emphasizing the scope of the various methods, their interrelations, and how they fulfil the conditions necessary for satisfactory analysis. It avoids the pitfall of proofs of propositions and distributions, as well as that of being too technical, yet to some it may seem so. One cannot too strongly urge students of biological problems to become familiar with the sign language used in such work. The book is graded to build up to the more severe mathematical requirements in the later chapters. The subject is covered in twelve chapters, as follows: Introductory; probability and significance; distributions; tests of significance; the significance of single observations, sums, differences, and means; degrees of freedom and the analysis of variance; planning experiments; the